PHARMACEUTICAL SCIENCES

ESTABLISHMENT OF SOME PHARMACEUTICAL STANDARDS FOR NIGERIAN MEDICINAL PLANTS I: *FAGARA* LINN. SPECIES

By

A. O. ADEOYE and E. A. SOFOWORA

Department of Pharmacognosy
Faculty of Pharmacy, University of Ife,
Ile-Ife, Nigeria.

SUMMARY

Specimens (obtained from various collection sites) of each of three common *Fagara* Linn. Species were exa-mined with a view to setting some pharmacopoeial standards* including percentage total ash, acid-isoluble ash, water-soluble as as well as perventage soluble extractives. Fixed values for commercial samples were recommended, based on the experimental findings. The significance of the findings are discussed in relation to our recommendations for standards to be included in a local pharmacopoeia in respect of these crude drugs.

Recently, there has been a renewed interest in phyto-therapy due to precise phytochemical and biological screenings which show the potentials of tropical medicinal plants in health care delivery system of the developing countries of the world. The roots of *Fagara* Linn. species have been implicated as possessing great potentials in phytotherapy by their antisickling, antimicrobial and antitumor activities (EL-SAID *et al.* 1971; SOFOWORA and Isaacs 1971; Messmer *et al.*, 1972; ISAACS-SODEYE *et al.*, 1975; HEADINGS *et al.*, 1972; ODEBIYI and SOFOWORA; 1978;). The need to control the quality of vegetable drugs has been highlighted in various pharmaco-poeias and recently at conferences on utilisation of medicinal plants. Our present series of articles are aimed at contributing some data to the quality control of vegetable drugs in tropical Africa particularly those drugs for which no standards are available and which continue to be consumed for medicinal purposes in this part of the world.

MATERIALS AND METHODS

Materials and authentication

All root specimens were collected between October 1977 and April 1978 in the southern part of Nigeria, mostly from moist lowland rain forest where these three species of *Fagara* studied are found. Four separate collections (about 5—10kg each) were made for each of *F. macrophylla* Engl., *F. tessmannii* Engl., and *F. zanthoxyloides*. The roots were authenticated by Dr. Olatunji (Department of Biology, University of Ife) and voucher specimens were deposited with the University of Ife Herbarium.

Preparation of Material

Adherent soil was shaken off from the roots and the latter were divided into two portions. One portion was freed from soil under running tap water with the aid of a small brush. This afforded "washed" roots. The other untreated portion afforded "unwashed" roots. The two grades of roots were separately chopped into small pieces and dried in an hot-air oven at 60° before communiting them to coarse powder grade of the BRITISH PHARMACOPOEIA (1973A) unless other-wise specifled.

Sampling: Representative samples of root were taken from each of the four collections made for each species. Twelve replicates (from each of four representative sample) were used for each of the estimations reported below except in the case of percentage acid-insoluble ash and water-soluble ash determinations where six replicates were used for each.

Determination of ash values

Total ash: A known weight, W, (3 to 5g) of coarsely pow-dered "washed" or "unwashed" root was accurately weighed into a clean tared porcelain crucible. The powder was incinerated in a muffle furnace[1] following the procedure described in the BRITISH PHARMACOPOEIA, (1973B). Complete ashing was effected after 8 hr. The crucible and its contents were dried to constant weight and the weight of total ash (T) determined by difference. Percentage total ash was calculated as follows:

[1]Carbolite (R) Electrical Furnace Model 7/76/592 was used.

$$\text{Percentage total ash} = \frac{T \times 100}{W}\%$$

Acid-insoluble ash: The total ash (T) obtained was dissolved in 25ml dilute HCl (0·01N) and gently boiled over an open bunsen flame. The ash-acid mixture was allowed to cool before it was filtered through an ashless, fluted filter paper. After washing the residual ash with freshly distilled water to free it from acid, the residual ash (together with filter paper) was placed in a tared crucible and dried at 80°C for 30 min. The crucible and its contents were then incinera-ted in the furnace (BRITISH PHARMACOPOEIA, 1973b). Complete ashing was effected in 3hr. after which period the crucible and its contents were dried to constant weight. The acid-insoluble ash (A) was obtained by difference. Percent-age acid-insoluble ash was calculated as follows:

$$\text{Percentage acid-insoluble ash} = \frac{A \times 100}{W}$$

Water-soluble ash: The total ash (T) was dissolved in 25ml of freshly distilled water, gently boiled over an open bunsen flame, allowed to cool and then filtered using an ashless fluted filter paper. The residual ash (S) was dried at 80°C for 30 min before incineration in the furnace. Complete ashing was obtained after 3hr. and, after cooling, the residual ash (S) was dried to constant weight. The weight of water-soluble ash was obtained by differences (T-S) and the percentage water-soluble ash calculated as follows:

$$\text{Percentage water-soluble ash} = \frac{(T-S) \times 100}{W}\%$$

Determination of soluble extractive values

The following determinations were carried out using coarse and moderately—coarse grades (BRITISH PHARMACOPOEIA, 1973a) of powdered roots. The solvents used were *0·5% chloroform water B.P.* and 80% ethanol for water-soluble and alcohol-soluble extractives respectively.

5g of powdered root was accurately weighed into a clean conical flask. 100ml of solvent was added to the powder and the flask was stoppered. With the aid of a magnetic stirrer, the mixture was stirred intermittently for 6hrs. and allowed to stand for 18hrs after which period the mixture was filtered under suction through a Buchner funnel. The filtrate was measured in 20ml aliquots into dry tared procelain crucibles (BRITISH PHARMACOPOEIA, 1973c). The crucibles and their contents were heated to dryness in an oven at 105°C for 1½ to 2hrs. After cooling, the weight of the soluble extractive was noted and the percentage soluble extractive calculated as follows:

$$\text{Percentage soluble extractive} = \frac{We \times Vs \times 100}{Va \times W}\%$$

where We = Weight of extract obtained from aliquot (g)
Vs = Total volume of solvent used to extract (ml)
Va = Volume of aliquot taken (ml)
W = Weight of air-dried drug taken (g)

Estimation of total acids

In estimating the total acids contained in the antisickling fractior of *Fagara* Linn. roots as p-hydroxybenzoic acid, the procedure described by ELUJOBA and SOFOWORA (1977) was adopted.

RESULTS AND DISCUSSION

Table I shows the results obtained for per cent, total ash, acid-insoluble ash and water-soluble ash for the three species of Fagara Linn Most common in Southern Nigeria. **The total ash values for "washed" roots in the three species do not show any significant difference statistically. A similar situation was found in the case of acid-insoluble ash values. This was irrespective of the fact that the specimens used were collected from different geographical locations varying from forest savannah to mangrove swamp forest.**

However, the water-soluble ash value for "washed" roots of *F. zanthoxyloides* (0·38%) was consistently and significantly different from those obtained for *F. macrophylla* and *F. tessmannii*. These latter two species had similar (0·75% and 0·72% respectively) water-soluble ash values. The same species were also grouped together chemotaxonomically by FISH and WATERMAN (1973) and have been mistaken for each other until recently (OLATUNJI, 1976). The ash values for "unwashed" roots of the three species are not only higher than the corresponding values for "washed" roots (where adherent soil has been removed) but also differ significantly from one another as expected since the adherent earthy matter on the "unwashed" roots are in diverse quantities considering the variety of soil type location of the plant. It is therefore recommended that "washed" roots are better employed for evaluating these pharmacopoeial requirements in commercial samples of Fagara Linn. roots.

Table I
Ash values for *Fagara* roots

Species	*"Washed" Roots*			*"Unwashed" Roots*		
	**Total ash (Per cent)*	***Acid-insoluble ash (Per cent)*	***Water-soluble ash (Per cent)*	**Total Ash (Per cent)*	***Acid-insoluble ash (Per cent)*	***Water-soluble ash (Per cent)*
F. macrophylla	4.40±0.27	2.49±0.32	0.75±0.07	9.36±0.46	8.06±0.53	0.50±0.06
F. tessmannii	4.15±0.24	2.75±0.24	0.72±0.06	6.84±0.30	5.47±0.48	0.32±0.03
F. zanthoxyloides	4.34±0.15	2.77±0.4	0.38±0.04	5.08±0.48	3.22±0.39	0.43±0.03

* Mean of 12 replicates for each of four different collections
** Mean of 6 replicates for each of four different collections.

Table II
Soluble extractive values for *Fagara* roots

Species	**Coarse Powder B.P.*		**Moderately coarse powder B.P.*	
	***Water (Per cent)*	****Alcohol (Per cent)*	***Water (Per cent)*	****Alcohol (Per cent)*
F. macrophylla	10.1±0.17	9.1±0.22	11.4±0.33	9.6±0.16
F. tessmannii	9.7±0.15	10.6±0.34	10.2±0.23	11.3±0.24
F. zanthoxyloides	12.3±0.23	8.5±0.24	10.2±0.96	11.4±0.16

*Mean of 12 replicates for each of four different collections
**0.5% Chloroform-water
***80% Ethanol

From the results obtained for the soluble extractive values (Table II), it would appear that a higher yield to both solvents (0.5% $CHCl_3$-water and 80% ethanol) was obtained in the case of moderately-coarse powder when compared with the yield from coarse powder in any given species. This is because a better communition was obtained in moderately-coarse powder and hence better solvent penetration. Across the three species however, the degree of communition had no effect on the minimum yield of water-soluble extractive value which is about 9·5% in both coarse and moderately-coarse powders. Alcohol gave a higher extractive value than water as expected, because it has a better penetrating and solvent ability during extraction. However, the determination of both water-soluble and alcohol-soluble extractive values is to be recommended in the quality control of these *Fagara* roots since the biological activities for which the roots are required have been reported present in both aqueous and alcoholic extracts (EL-SAID *et al.*, 1971; SOFOWORA and ISAACS, 1971; ODEBIYI and SOFOWORA 1978; MESSMER *et al.*, 1972; FADULU, 1975).

Table III
Total acids in antisickling fraction of *Fagara* roots

	TOTAL ACIDS (%$^{w}/_{w}$**)
F. macrophylla	0.45±0.04
F. tessmannii	0.37±0.02
F. zanthoxyloides	0.15±0.04

* Calculated as p-hydroxybenzoic acid.
** Mean of 16 replicates for each of 4 different collections.

Each of the *Fagara* root specimens examined was found to contain acids in the antisickling fraction as earlier reported by ELUJOBA and SOFOWORA (1977). It was also observed that the interspecies variation in the total acid value was significant (Table III) but we are of the opinion that with respect to the antisickling activity of these common *Fagara* Linn. roots, any root specimen having not less than 0·1%$^{w}/_{w}$ total acids when estimated by the method of ELUJOBA and SOFOWORA (1977) should be commercially acceptable.

The following analytical standards for "washed" roots of *F. macrophylla, F. tessmannii* and *F. zanthoxyloides* are recommended, based on our results, for inclusion in pharmacopoeial monographs for use in evaluating the quality of commercial samples of *Fagara* Linn. roots:—

Total ash—not more than 5·0%
Acid-insoluble ash—not more than 3·0%
Water-soluble ash—not less than 0·3%
Water-soluble extractive
  Not less than 9·0% (Coarse powder)
  Not less than 10·0% (Moderately-coarse powder)
(80%) Alcohol - soluble extractive
  Not less than 8·0% (Coarse powder)
  Not less than 10·0% (Moderately-coarse powder)

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of the binding. Thus cyanide, with a dissociation constant of about 10^{-6}M gives a Hill coefficient of about 2·5, whereas azide with the much larger dissociation constant of 0·1 millimolar shows no cooperative interaction at all.

SPECIES VARIATION IN THE METABOLISM OF 1-NAPHTHYLACETYLGLYCYLGLYCYLGLYCINE

By

P. A. F. DIXON

Department of Pharmacology, Faculty of Pharmacy, University of Ife.

1-Naphthylacetylglycylglycylglycine was metabolised in rat and rabbit to mainly 1-naphthylacetylglucuronide; in toad, to 1-naphthylacetic acid and 1-naphthylacetylglycine, and in Lizzard and tortoise to 1-naphthylacetic acid and its glycine and ornithine conjugates. In the *in vitro* studies there were varying amount of 1-naphythylacetic acid, its glycine and glycylglycine conjugates and some of the unchanged compound in all the species.

STUDIES ON THE GIANT AFRICAN SNAIL ACHANTINA FULICA

By

S. O. A. BAMGBOSE

Department of Pharmacology, College of Medicine University of Lagos.

Our interest in the Giant African Snail *Achantina fulica* started after the controversy over its effectiveness in the cure of hypertension (Tella 1973, Mabadeye 1973). We were not interested in its action in hypertension but in doing some systematic work on some pharmacological and biochemical properties of the snail, we have shown that the tissue of the African snail react best to drugs in modified Meng's solution and that there is some evidence of cholinergic innervation in the smooth muscle. Our study has shown some similarity in the response of the tissues to the mammalian tissue. Evidence for sympathetic innervation has not so far been found. The intestine of the Giant African Snail is made up of both smooth and skeletal muscles (Bamgbose 1976).

We have also examined the properties of the haemolymph (Adedevoh, Bamgbose, Olatunbosun and Ogbinu 1976; Bamgbose, Elegbe and Kareem). Some of our results agree with those of Aboderin and Kareem (1971) although we found three peaks on Sephadex Co-200 similar to 19s, 7S and 4·5S of human while the total protein concentration ranged from 1·5-5·9 gms/100ml of haemolymph. There was no cross reaction between the snail haemolymph and human anti-kappa and anti-hamoda sera in the onchtestony test.

The haennolymph which was shown to possess anti-trypsin activity (Kareem 1974) also exhibited anti-inflammatory properties (Bamgbose, Elegbe & Kareem 1974). It is however inferior to chlorpromazine in this respect and might likely be acting by inhibiting the release of autocoids.

REGIONAL VARIATION OF THE FRUIT-EATING BAT'S GUT TO CHOLINERGICS

By

A. O. ADEBANJO

Department of Pharmacology, University of Ife.

Plexus-containing longitudinal or plexus-containing tubar preparations of 4 different regions of the gastro-intestinal tract of the bat (Eidolon helvum) were exposed to some cholinergic agents under constant physiological conditions. A considerable differentiation of the responsiveness to these agents was observed in experiments involving more than 100 bats of either sex.

The results clearly suggest the followings:

(*i*) An abundance of acetylcholine receptors as displayed by the intense susceptibility to small doses of acetylcholine (threshold doses of 10^{-12}g/ml; 5·5pM) for the ileum, 10^{-10}g/ml; 0·55nM) for the duodenum and 10^{-7}glml; 0·55nm) for either the oesophagus or the rectum.

(*ii*) The presence of ganglion cells in three regions as shown by nicotine-induced contractions or relaxations.

(*iii*) An interference by intrinsic pendular movements in all regions save the rectum and a reversal of this contamination by indomethacin 10^{-7}—10^{-5}g/ml implicating prostaglandins in the mediation of these movements.

(*vi*) An ease of abolition of these cholinergic effects with minute doses of atropine 10^{-10}—10^{-8} g/ml (0·29—29nM).

RELEASE OF PGE_2 FROM ISOLATED PERFUSED LIZARD LUNG AND ITS USE FOR ASSAY OF PROSTAGLANDIN SYNTHETASE INHIBITORS

By

F. O. OLADITAN & D. T. OKPAKO

Department of Pharmacology & Therapeutics University of Ibadan.

It has been shown previously that high amounts of PG-like material can be extracted from the lungs of the Rainbow Lizard (Okpako, 1976).

We have extended this study. Male and female Lizards were decapitated and pithed. Heart and lungs were isolated and transferred to a dissecting surface. A flexible polythene cannula (i.d. 1mm) was inserted in the main pulmonary arterial trunk through an incision in the ventricle. The pulmonary vasculature was perfused with aerated frog Ringer at 31°C (5 ml min^{-1}). The perfusate effluent was used to superfuse prostaglandin sensitive assay tissues-rat stomach strip, rat colon and chick rectum —treated with a combination of antagonists to prevent effects due to histamine, acetylcholine, 5-hydroxytryptamine and catecholamines.

The perfusate effluent contracted all the assay tissues. Activity in the effluent declined from about 12ng ml^{-1} PGE_2 equivalent in the fraction collected 20min after the start of perfusion to 2ng ml^{-1} in fractions collected 60min later. Large amounts (50-75ng ml^{-1}) could be released repeatedly by 'stroking' (gently rolling a glass rod over the surface of the lung). 98% of this activity was identified as PGE_2 by thin-layer chromatography and differential bioassay. The release was characteristically blocked by the prostaglandin synthetase inhibitors, aspirin indomethacin, ketoprofen, sodium meclofenamate, and benaxoprofen with activity ratios reflecting their anti-inflammatory potencies. Cortisone at all doses tested enhanced the release of PGE_2. The value of this model in rapid screening for PG synthetase inhibitor activity will be discussed.

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MUSCARINIC RECEPTORS IN THE ISOLATED RECTUM OF THE WEST AFRICAN LAND TORTOISE (KINIXYS SPP)

By

J. O. A. OJEWOLE,
Department of Pharmacology,
Faculty of Pharmacy, University of Ife,
Ile-Ife, Nigeria.

In recent years, studies on the pharmacology of the various smooth muscle preparations isolated from our local common reptiles (e.g. the rainbow lizard—*Agama agama*), and moluscs [*Achatina fulica*) have engaged the attention of many Nigerian investigators (Bamgbose, 1976; Fabiyi and Okpako, 1973, and, Marquis and Odebode, 1978). As a further contribution to the present knowledge on the pharmacology of West African non-mammals my attention has been focused on the tortoise which is another common West African land reptile. The present paper describes the action of some drugs on the isolated rectum of the tortoise.

Segments (2–3 cm long) were obtained from isolated rectum of male and female West African land tortoises (*Kinixys* Spp) weighing between 150 and 780g. The tissues were separately suspended in 10ml organ baths containing Krebs (1950) solution with double glucose (continuously bubbled with atmospheric air and maintained at 32°C under an applied resting tension of approximately 1g). The responses of the tissue preparation to drugs were recorded isotonically on smoked drums.

The following results were obtained:

(1) The isolated rectum of the tortoise [*Kinixys crosa or K. homeona*) contracted in response to acetylcholine, acetyl-B-methylcholine, arecoline, pilocapine and carbachol, but not to nicotinic stimulant (i.e. nicotine, DMPP or TMA), histamine, 5-HT, or sympathomietic drugs.

(2) the isolated preparation is sensitive most to acetylcholine, and the effect of the latter drug can be easily inhibited or abolished by exogenous addition of atropine to the bath fluid,

(3) A linear relationship always existed between the log (dose ratio-1) of acetylcholine and the negative log molar concentration of the antagonist (atropine).

(4) The mean pA2 value of atropine against acetylcholine was found to be 9·55 ± 0·24.

From these results, the acetylcholine receptors of the tortoise isolated rectum were classified as "muscarinic" (a) since the contractions induced by acetylcholine were markedly antagonised by atropine, but not by hexamthonium or d-tubocurarine; (b) atropine exhibited characteristics of "competitive antagonism" on the dose/response curves of acetylcholine; and (c) atropine showed a high mean pA_2 value against acetylcholine.

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