

ANTI-ASTHMATIC ACTIVITY OF WATER EXTRACT OF ROOT OF *Garcinia kola*

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ABSTRACT

*The root of *Garcinia kola* was collected at Kasuwar Bacci, Kaduna state, on 10th December, 2003. A root part of the plant was air dried, grounded to powder and then percolated using ethanol (98%). The crude extract was then partitioned with chloroform and water. The water extract was subjected to thin layer chromatography, two components (A and B) were observed, column chromatography was applied, and two components were separated. Both components were tested against tracheal rings of guinea pig and showed a positive result for bronchodilation.*

KEYWORDS: *Garcinia kola, crude extract, water extract, tracheal rings, bronchodilation.*

INTRODUCTION

Historically, natural products provide the oldest source for modern drugs synthesis. Natural selection during evaluation and competition between species has produced powerful biologically active natural products which serve as chemical leads, refined by chemist to give more specifically acting drugs. (Rufa'i, 2001).

Medicinal plants contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. (Sofowora A. 1982).

Garcinia Kola (bitter kola) "*namijin goro*" in Hausa and "*epa oro*" in ebira (zach o. obile vernacular names), widely spread to Northern part of Nigeria is commonly found in southern Nigeria due to its local and pharmaceutical uses. (Rufa'i,2001).

The tree is 20-30 feet high with shining leaves (3-6 inches). The seed is about 3.5 cm long and 1.5 cm wide. (Rufa' I Y. 2001)

The nut is chewed as kola adjuvant as well as a true kola, it prevents or relieves cough, horsiness and improve singing voice. It lowers the blood pressure in pregnant women (mostly in southern Nigeria). It is used in the treatment of headache and dysentery. The nut is also found to be effective in treatment of bronchitis and throat trouble when mixed with other plants (Rufa' I Y. 2001).

Asthma is a chronic obstructive condition, it is not considered as a part of chronic obstructive pulmonary disease as this term refers specifically to combination of disease that are obstructive such as bronchiectasis, chronic bronchitis and emphysema.(Timothy, 2009).

The study was carried out with objective of determining the water extract of the root of bitter kola and its activity on the bronchial asthma using the tracheal part of guinea pig.

MATERIALS AND METHODS

The root of *Garcinia kola* was collected from Kaduna state, Kasuwar Bacci market and was identified at The Department of Biological science, BUK by m. Ali Garko (the chief gardener)

EXTRACTION PROCEDURE

The root of *Garcinia kola* was air dried, grounded with pestle and mortar and sieved into fine powder. 130g of the powdered sample was percolated with 1.7L of ethanol (98%). The

solvent was distilled on a Buchi rotary evaporator. The residue was weighted and labeled R1.

PARTITIONING OF THE RESIDUE (R1)

R1 was partitioned between water and chloroform (40ml, 1:1) three layers were formed i.e. chloroform layer, interface layer and water layer. The chloroform, interface and water layer were separated using the separatory funnel, weighted R2, R3 and R4 respectively. (Rufa'i. 2001)

THIN LAYER CHROMATOGRAPHY ANALYSIS OF THE WATER EXTRACT (R4)

The practice of thin layer chromatography is separated into three distinct operations as follows:-

Ten gram(10g) of the adsorbent silica gel (size 20-25microns) was shake with 20ml of distilled water for three minute in a 250ml beaker and was sprayed by the use of spray gun on to the plates (15cm by 7cm). the plates were allowed to stand undisturbed, dried for 5-10minutes before they were oven dried at 100 °C for 15-20minutes. Exactly 0.5mg of the extract was dissolved in water to make a solution of the extract. The solution was spotted by means of micro capillary glass tube at about 1.5cm from the bottom of the chromo plate. The spots were allowed to dry, after been dried, a straight line was marked in such a way that the line did not damage the coating (John, 1956).

Ethanol was used as a solvent for this thin layer chromatography (TLC) were ethanol (98%) and chloroform 40ml (1:1), poured into a glass jar with a glass plate as a cover. The chromo plates was slide on the wall of the glass jar dipped in the solvents vertically, and the spots were allowed to ascend upward from the origin for about 5 minutes after which the chromo plates were removed and the solvent front was marked with a fine pencil point. The distance moved by the substance and the distance moved by solvent front were measured for the calculation of retardation factor (R_f). The chromo plate was subjected to iodine vapour in a glass tank for 10 mins, to develop the spot, and observed clearly that there are two components in the extract (William. 1967).

COLUMN CHROMATOGRAPHY ANALYSIS

(i) **Packing the column:**

The slurry was prepared by dissolving about 600g of silica gel (60-120 mesh) in the ethanol. The slurry of the adsorbent was poured into the column carefully by the use of glass Funnel, the column was tapped using wooden rod to remove the air spaces inside the slurry of adsorbent, making an even distribution of the slurry in the column. (DuPont, 1980).

(ii) **Addition or loading the sample**

The extract was dissolved in water being it is water extract, applied on to the top of the adsorbent by means of capillary glass pipette distributed evenly and carefully, chloroform as the other solvent was poured carefully and gently to the column in such a way that the adsorbent

and the loaded sample were not disturbed. (DuPont, 1980).

(iii) **Elution of the sample**

The column was developed by gravity flow with solvent head (chloroform) between 3-5cm. This is conveniently achieved by the chloroform solvent in a dropping funnel, closed with a stopper at the mouth of the column. (Vogel, 1948).

The components were collected in small beakers at time interval of 6-7mins, about 6 portions were collected one after the other. Later another TLC was carried out, it allowed mixing the component with the same characteristic TLC, hence, 2 different components were separated and labeled "component A" and "Component B" (DuPont, 1980).

Table1. Reagents used.

Reagents	Weight/volume
NaCl	60g
KCl	35ml
MgSO ₄ .7H ₂ O	29ml
KH ₂ PO ₄	16ml
Glucose	20g
NaHCO ₃	21g
CaCl ₂	25.2ml
Aerating gas	O ₂ 95% + CO ₂ 5%

BIOASSAY

Physiological salt solution (Krebs solution) was carefully prepared by taking an accurate weight of reagents into volumetric flask (10 liters) using analar grade reagent, distilled water was added and shaken thoroughly to make 10 liters. The Krebs solutions is mainly used for mammalian skeletal muscle experiment analysis, it keeps the tissue of fresh killed animal alive for experimental test (Rufa'i, 2001).

PHARMACOLOGICAL TESTING USING ISOLATED GUINEA PIG TRACHEAL RINGS

Guinea pig weight 300 - 500g and free from diseases were chosen for the experiment. Each guinea pig was killed by sharp blow on the head. Throat cutting as near to the head as possible was made, and it was at once transferred into a dish containing Krebs solution. It was cut transversely between each segment of cartilage so as to give a number of tracheal muscles. Six of the tracheal rings were tied together using thread and needle with cotton to form a chain and then mounted in Krebs solution at 37 °C and aerated with a mixture of oxygen (95%) and carbon (iv) oxide (5%) in an organ bath (15ml), one end of the chain was attached to a fixed pin inside the organ bath and the other end to a lever recording response on a smoke drum. (Rufa'l. 2001).

A control experiment was performed by maintaining the tracheal tissue rings in Krebs solution in organ bath for 5 minutes. A solution of salbutamol (ventoline) was prepared by dissolving 2mg in 10ml of normal saline which

made a solution of 0.2mg/ml. Thus 0.2 ml, 0.4ml, 0.6ml and 1.0ml of 0.2mg/ml ventoline were injected in separate experiment into the organ bath containing the tracheal rings in Krebs solution. Each experiment was monitored for 5 minutes in order to observe any response of the tracheal muscle on the smoke drum.

Also "component A" was prepared by dissolving 0.002g of component "A" in 2ml of normal saline which made a solution of 0.010g i.e. 10g/ml of component A. thus, 0.4ml of 10g/ml "component A" solution was injected in separate experiment into the organ bath containing the tracheal rings in Krebs solution. Each experiment was monitored for 5 minutes and observed any response on the smoke drum. All responses were recorded. The same experiment was performed using "component B" and all the responses showed by the "component B" were recorded in similar way. (Rufa'l . 2001).

RESULTS

The TLC analysis showed that the water extract contain 2 components "A" and "B". the component "A" was found viscous, sticky, dark brown in colour, and distance moved by the spot is 7.0cm, distance moved by the solvent front is 11.0cm, the Rf is 0.6cm while the component "B" was found viscous, sticky, brown in colour, and distance moved by the spot is 6.0cm, distance moved by the solvent front is 11.0cm, the Rf is 0.5cm. Both the components (A and B) were active against bronchial asthma as shown in table 4 and 5.

Table 2. fraction with its weight

SOLVNT USED(ml)	WEIGHT (g)
Water extract	1.2
Component "A"	0.43
Component "B"	0.51

Table 3. Relaxation response (cm) of bronchial muscles from ventoline tablets.

VENTOLINE	CONCENTRATION(g/ml)	RESPONSE(cm)
1	0.0080	0.010
2	0.0016	0.20
3	0.0024	0.25
4	0.0032	0.35
5	0.0040	0.45

Table 4. Relaxation response (cm) of bronchial muscles from component "A"

COMPONENT "A"	CONCENTRATION(g/ml)	RESPONSE(cm)
1	0.0040	0.05
2	0.0044	0.08
3	0.0033	0.13
4	0.0053	0.20

Table 5. Relaxation response (cm) of bronchial muscles from component "B"

COMPONENT "A"	CONCENTRATION(g/ml)	RESPONSE(cm)
1	0.0040	0.02
2	0.0044	0.25
3	0.0033	0.35
4	0.0053	0.40

DISCUSSION

When component "A" was used in the experiment the bronchial smooth muscle relaxed as shown by the signal recorded on the smoke drum, while, when the component "B" was subjected to the experiment, the relaxation of the tracheal smooth muscle was higher. This showed that the component "B" is more potent than the component "A". This showed that "A" is twice effective than "B".

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CONCLUSION

The component A & B of the water extract from the root of *Garcinia kola* exhibit bronchodilation activity on the tracheal smooth muscle in the treatment of asthma using an organ bath containing Krebs's solution and salbutamol I(ventoline) as a control.

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