Chemical Constituents and *In Vitro* Antimicrobial Activities of Five Botanicals Used Traditionally for the Treatment of Neonatal Jaundice in Ibadan, Nigeria

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Abstract: Neonatal jaundice is a main cause of morbidity and mortality among neonates in Nigeria and ethnobotanical investigation revealed the use of *Alstonia boonei* (De wild.), *Cocos nucifera* (L.), *Gossypium barbadense* (L.), *Tetrapleura tetraptera* (Schumach. & Thonn.) and *Vernonia amygdalina* (Del.) for the management of neonatal jaundice in Ibadan, Nigeria. This study analysed the five plants for their chemical components and antimicrobial activities to justify their therapeutic values in the management of jaundice. The powdered plant samples were screened for mineral and phytochemical components using standard protocols. The ethanol extracts (50 mg/ml) of samples were tested against five clinical pathogenic organisms at 10^{-1} – 10^{-6} cfu/ml inoculum concentrations using agar-well diffusion method. Data were analysed statistically. *G. barbadense* (0.26 mg/100g) had the highest Na content. *A. boonei* had the highest Zn and Fe contents, 6.40 and 5.13 mg/100g respectively. *A. boonei* contained the highest saponins (0.79%) and tannins (0.05%). At 1 x 10^{-4} cfu/ml, *G. barbadense* was most active on *K. pneumoniae* (29.90 mm); *T. tetraptera* was most active against *C. albicans* (21.90 mm) and *T. tetraptera* and *V. amygdalina* gave the same activity (14.90 mm) against *E. coli.* At 1 x 10^{-6}cfu/ml, only *T. tetraptera* showed 100% antimicrobial activity by being active against all test organisms. The mineral and phytochemical components of the plants could be responsible for their antimicrobial properties. The extracts, compounds as well as oils from the test plants especially *A. boonei* could be useful in combating infections and metabolic disorder associated with neonatal jaundice. Toxicity studies of the plant samples would ascertain their safety in treatments.

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1. Introduction

Hyperbilirubinemia is the medical term for a high bilirubin level in the bloodstream and the cause of jaundice (Coleman, 2011). Jaundice itself is not a disease, but rather a sign of one of the many possible underlying pathological processes that occur at some point along the normal physiological pathway of bilirubin metabolism. Hyperbilirubinemia is often seen in liver diseases such as hepatitis or liver cancer. It may also indicate obstruction of the biliary tract, for example by gallstones or pancreatic cancer, or less commonly congenital in origin. Jaundice can also be associated with severe illnesses such as haemolytic disease, metabolic and endocrine disorders, enzymatic deficiencies of the liver, and infections (Bilgen *et al*, 2006).

Neonatal jaundice is the most common form of jaundice and it occurs in new-born babies of less than a month old. Factors influencing the occurrence of neonatal jaundice are body physiology, premature birth, effect of breastfeeding, breast-milk constituents, heredity (enzyme deficiency), blood or rhesus group incompatibility (between mother and baby), and infections (Kotal *et al*, 1996; Bilgen *et al*, 2006; Kumral *et al*, 2009; American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004).

Clinical symptoms of neonatal jaundice include irritability, jitteriness, increased high-pitched crying, lethargy and poor feeding; back arching; apnea and seizures (Nelson, 2013). Phototherapy is the most effective way of breaking down a neonate's bilirubin (Leung *et al*, 1992) and increased feedings also help move bilirubin through the neonate's metabolic system. Exchange transfusions are also useful in the treatment of neonatal jaundice. (American Academy of Pediatrics Subcommittee on Hyperbilirubinemia, 2004).

Neonatal jaundice associated with high morbidity and mortality is a common pediatric problem in West Africa (Sofoluwe and Gans, 1960). The condition is the commonest cause of neonatal admission to Children Emergency Room in Lagos University Teaching Hospital (LUTH), Nigeria (Ransome-Kuti, 1972). In a study on the incidence and causes of neonatal jaundice in Nigerian babies, Effiong *et al*. (1975) observed that G6PD (enzyme glucose-6-phosphate dehydrogenase) deficiency and ABO incompatibility were the major aetiological factors in babies with total bilirubin of 15mg/100ml.

Ahmed *et al*. (1995) reported septicaemia (50%) and G6PD deficiency (40%) as the major aetiological factors of neonatal jaundice in 587 neonates born in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Nigeria. Neonatal jaundice accounted for 35% of all Neonate Intensive Care Unit (NICU) admissions at Federal Medical Centre, Abakaliki, Southeast, Nigeria. Septicaemia (32.5%) and prematurity (17.5%) were the leading aetiological factors in neonates (Onyearugha *et al*., 2011).

In view of non-drug orthodox methods of treatment of neonatal jaundice and the prevalence of neonatal jaundice in Nigeria, a phytodrug therapy would be desirable. Medicinal plants with reported therapeutic effects in the management of neonatal jaundice are *Artemisia capillaris*, *Scutellaria baicalensis*, *Rheum officinale*, *Glycyrrhiza glabra* and *Coptis chinesis* (Fok, 2001); *Boswellia serrata* (Patwardhan *et al*., 2010); *Alstonia boonei* (Adotey *et al*., 2012; *Moronkola and Kunle*, 2012) and *Gossypium barbadense* (Todou and Konsa, 2011). Rhei rhizome, ginseng, grapeseed, *Dioscorea villosa*, *Pseudolarix kaempferi*, *Hypericum perforatum* and soy have also been reported to be effective in the management of neonatal jaundice (Lazar, 2004).

In Nigeria, the stem bark of *A. boonei* is used widely to treat malaria, typhoid fever, gonorrhoea, yaws, asthma, dysentery, and as a galactagogue. *C. nucifera* roots have antipyretic and diuretic properties. Milk of young coconut is useful as laxative, antidiarrhoeic and counteracts the effects of poison. The oil is used to treat diseased skin and teeth, and mixed with other medicines to make embrocations. *G. barbadense* is widely used for the treatment of conjunctivitis, convulsions, jaundice, gastrointestinal disorders, and sexually transmitted infections such as gonorrhea, rheumatism and wounds. *T. tetraptera* is used traditionally for the treatment of skin infections. *V. amygdalina* has antibiotic value in traditional medicines, it is also used to treat diabetes, gastrointestinal disorders and as a worm expeller (www.prota4u.org).

This study presents scientific information on the therapeutic potentials of *A. boonei*, *C. nucifera*, *G. barbadense*, *T. tetraptera* and *V. amygdalina* in the management of neonatal jaundice.

2. Material and Methods

Plant materials

Twenty herb-sellers of a local herbal market (Bode) in Ibadan, Nigeria were interviewed on traditional knowledge of management and treatment of neonatal jaundice. *Alstonia boonei*, *Cocos nucifera*, *Gossypium barbadense*, *Tetrapleura tetraptera* and *Vernonia amygdalina* were frequently mentioned in the twenty recipes collected from the herb-sellers. Herbal recipes were documented. The local name, parts of plant used, method of preparation and mode of administration were also recorded (Sofowora, 1982). The plant materials were purchased from a local herbal market. The samples were identified at species level in the University of Ibadan Herbarium (UIH). The plant samples were washed, dried (27^{o}C) and powdered. The powdered samples were stored (4^{o}C) in air-tight bottles for further use.

Mineral analysis of powdered plant samples

The methods of Walsh (1971) and AOAC (2005) were used for the mineral analysis of samples. After wet digestion, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) were analysed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bausch scientific AAS). Phosphorus was determined using Vanadomolybdate (Yellow method) (AOAC, 2005). Percentage transmittance was determined at 400 nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

Phytochemical screening of plant samples

Quantitative phytochemical analysis of powdered plant samples was carried out using AOAC (2005) methods.

Antimicrobial assay of ethanol plant extracts: The extracts (50 mg/ml) of samples prepared by cold extraction method in 80% ethanol, concentrated and used for the antimicrobial bioassay. The test organisms: *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were clinical isolates obtained from University College Hospital (UCH), Ibadan. The organisms were grown in nutrient broth for 18 h at 37^{o}C. Six different inoculum concentrations ($1 \times 10^{-1} – 1 \times 10^{-6}$ cfu/ml) of each isolate were prepared in sterile distilled water from the broth cultures via serial dilution. 1ml of the inoculum was thoroughly mixed with 19 ml of sterile nutrient agar and poured into sterile Petri dish. The agar was left to solidify. Two wells of 4 mm in diameter were punctured in each agar plate and 60 µl of each extract was filled into the wells with the aid of a sterile micropipette. 80 % ethanol was used instead of extract in the control experiment. Also plates containing the test organisms in agar without extract were used as control. All experiments were done aseptically and each experiment was replicated three times. The plates were incubated at 37^{o}C for 24 - 48 h. Readings were taken after 24 h and 48 h. The diameter of inhibition was measured in millimetres (mm).

Data analysis

Where applicable, data were statistically analysed using One-way Analysis of Variance (ANOVA) and expressed as mean ± SD. The Duncan

Multiple Range Test (DMRT) was used to test means for significance ($P < 0.05$).

3. Results and Discussion

The profile of the plant samples used in the management of neonatal jaundice is presented in Table 1. The plants belong to five different families: Apocynaceae, Arecaceae, Malvaceae, Fabaceae and Asteraceae. Table 2 shows the traditional methods of preparation of various botanicals as regimen for neonatal jaundice. The herbs are used singly in recipes and decoction is the most common method of preparation, the herbs are also prepared as soap, oil and syrup for administration. The folk methods of treatment take into consideration the fragility of the baby's body and system hence the mild methods of preparation and administration of herbal remedies.

The various mineral constituents of plant samples are presented in Table 3. *G. barbadense* (0.26mg/100g) gave the highest Na content and *C. nucifera* (0.04mg/100g) the least. K content was also highest in *G. barbadense* (0.48 mg/100g) and least in *T. tetraptera* (0.10 mg/100g). Calcium content was highest in *G. barbadense* (0.08 mg/100g) and least in *T. tetraptera* (0.01 mg/100g). *A. boonei* gave the highest P content of 0.41 mg/100g and *C. nucifera* (0.11 mg/100g) the least. Zinc (Zn) was highest in *A. boonei* (6.40mg/100g) and least in *C. nucifera* (3.25mg/100g). The iron (Fe) content of *A. boonei* (5.13mg/100g) was the highest and *T. tetraptera* (1.15 mg/100g) recorded the least. Overall, *G. barbadense* was highest in sodium, potassium and calcium. *A. boonei* also recorded highest values for phosphorus, zinc and iron. The results of the mineral constituents of test plants are in line with the reports of previous authors. Afolabi *et al*. (2007) reported the magnitude of presence of macroelements (ppm) in *A. boonei* bark in the order P>K>Na>Ca>Mg and only Fe (3.2 ppm) was detected as micronutrient. Although there is scarcity of information on the mineral constituents of coconut pod, the literature is replete with information on coconut water mineral components (Richter *et al*., 2005; Uphade *et al*., 2008). *G. barbadense* leaves contain copper (14.6 ppm) and zinc (1.6 ppm) (Apena *et al*., 2004). Essien *et al*. (1994) reported that the fruit shell, fruit pulp and seed of *T. tetraptera* contained varying amounts of nutrients such as protein, lipids and minerals. *V. amygdalina* contains calcium, iron, potassium, phosphorous, manganese, copper and cobalt in significant quantities (Eleyinmi *et al*., 2008). The various plant minerals have key roles to play in human health. Potassium regulates heartbeat, maintains fluid balance and helps muscle contraction. Calcium is important for developing and maintaining healthy bones and teeth. It assists in blood clotting, muscle contraction and nerve transmission. Phosphorus works in combination with calcium to develop and maintain strong bones and teeth. It also enhances the use of other nutrients. Magnesium helps nerves and muscles function. It activates over 100 enzymes in the body. Zinc is an essential part of more than 200 enzymes involved in digestion, metabolism and reproduction and wound healing. Copper helps in normal red-blood cell formation and connective tissue formation. It acts as a catalyst to store and release iron to help form hemoglobin. It also contributes to central nervous system function. Iron is essential for red blood cell formation and function and is very important for brain function (Schauss, 2013).

The five medicinal plants contained secondary metabolites in varied concentrations (Fig. 1). The % alkaloids was highest in *A. boonei* (0.47%), followed by *V. amygdalina* (0.19%), and the least was for *C. nucifera* and *G. barbadense* (0.16%). The % saponins was highest in *A. boonei* (0.79%), followed by *G. barbadense* (0.58%) and *C. nucifera* (0.26%) had the least. *A. boonei* and *V. amygdalina* had the highest tannins content of 0.05%. The % phenols was highest in *V. amygdalina* (0.08%). The highest glycosides content was observed in *T. tetraptera* (0.36%). Generally, *A. boonei* had the highest values of alkaloids, saponins and tannins.

At 1×10^{-4}cfu/ml inoculum concentration, the ethanol extract (50 mg/ml) of *A. boonei* was active on *E. coli* and *K. pneumoniae* with 11.90mm and 14.90mm zones of inhibition respectively (Fig. 2). *C. nucifera* extract was active on 2 out of 5 test organisms; it inhibited the growth of *S. aureus* (11.90mm) and *P. aeruginosa* (13.40mm). *G. barbadense* extract showed antimicrobial activities on 4 out of 5 isolates being most active on *K. pneumoniae* (29.90mm) and least active on *E. coli* (11.90mm). *T. tetraptera* fruit extract was active on three bacterial pathogens and *C. albicans*. The extract was most active against *C. albicans* (21.90mm) and least active on *S. aureus* (11.90mm). *V. amygdalina* extract had antimicrobial properties being most active on *K. pneumoniae* (23.40mm) and least active on *P. aeruginosa* (14.40mm).

Fig. 3 shows the antimicrobial effects of various plant extracts against organisms at 1×10^{-6} cfu/ml inoculum concentration. *E. coli* was susceptible to *C. nucifera* extract with 24.90 mm diameter of inhibition. The growth *K. pneumoniae* was inhibited by *T. tetraptera* extract with 44.90 mm zone of inhibition. Overall, at 1×10^{-6}cfu/ml inoculum concentration, only *T. tetraptera* showed 100% antimicrobial activity by being active against all organisms. In line with the results of the antimicrobial activities of the test plants are previous reports on their values as antibiotic plants. The antimicrobial activity of extracts and active compounds of *V. amygdalina* has been reported by previous authors

(Alabi *et al*., 2005; Erasto *et al*., 2006). Gbadamosi and Oyedele (2012) reported the antimicrobial activities of *A. boonei* and *T. tetraptera* in skin infections. The hot percolated ethanolic extract of *C. nucifera* was active against Gram + and Gram – bacterial isolates (Singla *et al*., 2011). *G. barbadense* has antibacterial and wound healing properties (Ikobi *et al*., 2012).

As neonatal jaundice is an indication that the baby's liver is limited in its ability to process bilirubin, the mineral and antioxidant constituents of the five plants may be therapeutically useful. According to White and Foster (2000) detoxification process of the liver initially requires magnesium, iron, molybdenum and essential fatty acids, then later extra vitamins A, C, E (antioxidants), zinc, copper, folic acid and some amino acids. *V. amygdalina* has antibiotic, antimicrobial, anticancer, antioxidant, antidiabetic, hepato-protective, nephro-protective, oxytocic and serum lipid modulation properties (Ijeh and Ejike, 2011). Also *T. tetraptera* has shown antimalarial and antioxidant properties (Lekana-Douki *et al*., 2011; Badu *et al*., 2012). Gbadamosi *et al*. (2011) reported the nutritional and phytochemical properties of *A. boonei* bark as an antimalaria herb. *C. nucifera* is rich in phosphorus and has antibacterial, antifungal, antiviral and antioxidant properties. The phytochemical components such as alkaloids, saponins and tannins present in the medicinal plants may have singular or synergistic effects in improving the ability of the neonate's liver to reduce high level of bilirubin. Since infection is a risk factor for neonatal jaundice (Bilgen *et al*., 2006) the antimicrobial activities of the medicinal plants will be useful in treating the health problem.

Table 1. Profile of test plants used in neonatal jaundice in Ibadan, Nigeria

Family	**Scientific name**	**Common name**	**Local name**	**Part used**
Apocynaceae	*Alstonia boonei*	Stool wood	Ahun	Bark
Arecaceae	*Cocos nucifera*	Coconut	Agbon	Pod (husk)
Malvaceae	*Gossypium barbadense*	Cotton	Owu	Leaf
Fabaceae	*Tetrapleura tetraptera*	-	Aidan	Pod (Fruit)
Asteraceae	*Vernonia amygdalina*	Bitter leaf	Ewuro	Leaf

Table 2. Herbal remedies for the management of neonatal jaundice in Ibadan, Nigeria

S/N	Herb	Herbal preparation and dosage	Preparation method
1.	*Alstonia boonei*	The bark (200g) of *A. boonei* is boiled in 1L of water for 15 mins. The water extract is used to bath the baby twice daily. Half teaspoonful of the extract is given to the baby orally twice daily after food.	Decoction
2.	*Cocos nucifera*	The pod (200g) of *C. nucifera* is cooked in 1L of water for 15mins and used to bath the baby. Powdered pod is soak in hot palm oil and menthol crystals are added to the oil. The oil is rubbed on the baby after bath.	Decoction and oil
3.	*Gossypium barbadense*	*G. barbadense* leaves (200g) are boiled in water (1L) for 15mins. One teaspoonful of the extract is given to the baby three times daily after food.	Decoction
4.	*Tetrapleura tetraptera*	*T. tetraptera* pod (200g) is cooked in water (1L) for 15 mins. The preparation is used to bath the baby twice daily. Half teaspoonful of the extract is taken by the baby twice daily after food. The powdered pod is mixed with local soap and used for bathing the baby twice daily.	Decoction and soap
5.	*Vernonia amygdalina*	Fresh leaves of *V. amygdalina* are collected and washed thoroughly. The leaf juice is extracted by pounding. The juice is added to pure honey (1:1) and mixed thoroughly. The baby takes one teaspoonful of the mixture three times daily. Paste is also prepared from the fresh leaves and mixed with local soap. The soap is used for bathing daily.	Syrup and soap

Table 3. Mineral components (mg/100g) of powdered plant samples

Botanical	Na	K	Ca	P	Mg	Zn	Cu	Fe
A boonei	*$0.12^{c} \pm 0.00$	$0.31^{c} \pm 0.00$	$0.06^{c} \pm 0.00$	$0.41^{a} \pm 0.00$	$0.49^{a} \pm 0.00$	*$6.40^{a} \pm 0.28$	$9.05^{a} \pm 0.35$	$5.13^{a} \pm 0.14$
C. nucifera	$0.04^{e} \pm 0.00$	$0.08^{e} \pm 0.00$	$0.03^{d} \pm 0.00$	$0.11^{e} \pm 0.00$	$0.23^{e} \pm 0.00$	$3.25^{e} \pm 0.21$	$4.35^{c} \pm 0.21$	$2.14^{d} \pm 0.21$
G. barbadense	$0.26^{a} \pm 0.00$	$0.48^{a} \pm 0.00$	$0.08^{a} \pm 0.00$	$0.39^{b} \pm 0.00$	$0.47^{b} \pm 0.00$	$5.63^{b} \pm 0.28$	$8.50^{a} \pm 0.28$	$3.88^{b} \pm 0.14$
T. tetraptera	$0.10^{d} \pm 0.00$	$0.12^{d} \pm 0.00$	$0.01^{e} \pm 0.00$	$0.26^{d} \pm 0.00$	$0.31^{d} \pm 0.00$	$3.66^{d} \pm 0.21$	$4.80^{c} \pm 0.14$	$1.15^{e} \pm 0.21$
V. amygdalina	$0.17^{b} \pm 0.00$	$0.34^{b} \pm 0.00$	$0.06^{b} \pm 0.00$	$0.29^{c} \pm 0.00$	$0.40^{c} \pm 0.00$	$4.28^{c} \pm 0.49$	$6.35^{b} \pm 0.21$	$3.15^{c} \pm 0.21$

Legend: *Value = Mean ± standard deviation. Values within a column followed by the same superscript are not significantly different at P = 0.05. Na - Sodium; K - Potassium; Ca - Calcium; P - Phosphorus; Mg- magnesium; Zn - Zinc; Cu - copper; Fe - Iron.

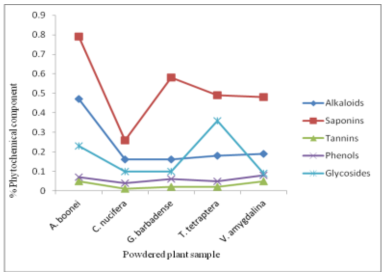

Figure1. Phytochemical components of powdered plant samples

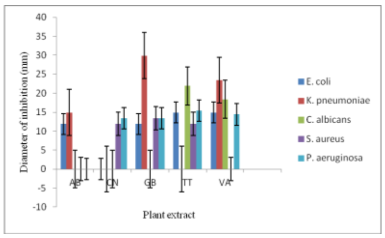

Figure 2. *In vitro* antimicrobial activities of ethanol extracts of test plants at 1 x 10^{-4} cfu/ml inoculum concentration

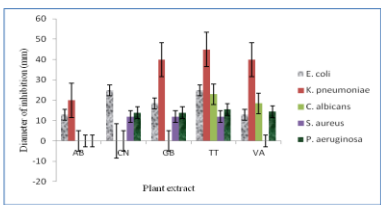

Figure 3. *In vitro* antimicrobial activities of ethanol extracts of test plants at 1 x 10^{-6} cfu/ml inoculum concentration

Conclusion

The test plants contained minerals and secondary metabolites that could be responsible for the observed antimicrobial activity of the plants. *A. boonei* is a valuable plant in the management of neonatal jaundice because of its high magnesium, copper, zinc and iron contents. Mg and Zn play significant roles in metabolism, copper and iron are essential in red blood formation and function. Enhanced metabolism flushes out bilurubin out of the baby's system. A decoction of *A. boonei* is good for the mother as galactogogue as well as for the baby for the treatment of jaundice especially in breast feeding and infections associated neonatal jaundice. Of importance is the significant antimicrobial activities of *G. barbadense* and *V. amygdalina* in infections associated neonatal jaundice. The isolation and purification of active components of *A. boonei*, *G. barbadense* and *V. amygdalina* could improve pharmaceutical treatments for neonatal jaundice. Toxicity studies of the three plants will certify their safety in treatments.

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