In-vitro and in-vivo antibacterial effect of Croton lobatus Linnaeus L. on two days post surgical wounds in rats

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Abstract

Phytochemical constituents of Croton lobatus L. (C. lobatus) water extracts and quantitative analysis were carried out following standard procedures. The antibacterial activity against Staphylococcus aureus (ATCC 33591); Streptococcus spp; Pseudomonas aeruginosa (ATCC 9028); Proteus vulgaris; Escherichia coli (ATCC 43895); and Salmonella spp (ATCC 4932) was carried out at the concentration of 0.5g/mL, 0.05 g/mL and 0.00 5g/mL of water. In vivo antimicrobial assay was carried out by creating four wounds of 0.5 by 0.5 cm on dorsal surface of a male albino rat under anesthesia. The wounds were left for 48 hrs, after which they were accessed and samples were collected for culture, identification and colony forming unit counts (CFU). Respective treatment using dried C. lobatus, C. lobatus (water extract), Physiological saline solution and Cicatrin powder was carried out and samples were collected at day one, three, five and seven after initiation of treatments for CFU counts on nutrient and MacConkey agar. The phytochemical studies revealed that C. lobatus contains carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids, alkaloids and tannins. Croton lobatus L. showed a dose dependent activity against micro organisms with C. lobatus 0.5 inhibited the growth of most bacteria at the zone of inhibition ≤ 21mm. This was also supported by in vivo antimicrobial assay. Secondary metabolite tannins, triterpenoids, flavonoids, crotonic acids and saponin were responsible for its antimicrobial activity against the tested microorganisms thereby supporting its usage by the traditional medicine practitioner in Nigeria to treat chronic wounds.

Keywords: Phytochemical screening; Antibacterial; Croton lobatus L.; Surgical wounds; Astraea lobata

Introduction

One thousand three hundred and thirty species of trees, shrubs and herbs belonging to Croton were distributed in the Northern and Southern Hemispheres (Block et al. 2004). Majority of which were popularly used for various purposes due to their medicinal qualities in different continents (Yibralig, 2007). Croton lobatus L. is commonly known as Russoil; Croton; Lobed croton (Malpighiales of North America Update, 2011). In Nigeria, Burkhill reported that the plant is known in three major languages which are: Gaásàyaá (Hausa); Òkwè-one a kind of bean (Igbo); Ajěiofólé or ọlù (Yoruba). The old name was Astraea lobata Klotzsch (Global Biodiversity Information Facility, 2016; Malpighiales of North America Update, 2011). Croton lobatus L. is native of Caribbean and American but introduced in different part of African countries (Gaikwad et al., 2012; Malpighiales of North America Update, 2011).

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In Africa, *Croton lobatus* is commonly used by Indigenous people to treat different diseases such as malaria, diarrhea and problems due to pregnancy (Aké, 1975). The crude extracts from the aerial and roots of *Croton lobatus* have anti-plasmodia activity (Weniger et al. 2004). Heated leaves are rubbed on to areas of costal and rheumatic pain, and a leaf-decoction by mouth, or a bark-decoction by enema is given as a purgative (Burkill, 1985). The plant is used generally as healing leaf medicine, pain-killer, laxative, cutaneous and subcutaneous parasitic infection, treatment for menstrual cycle disorders, antifertility (venomous stings and bites), treatment for paralysis, epilepsy, convulsions, spasm and topically to treat ulcers, sores, and headache (Burkill, 1985). Odukoya et al. (2012) reported that *Croton lobatus* is usually boiled with water and the decoction is taken to treat wounds that refuse to heal while Bouquet and Debray, (1974) reported the whole plant infusion to be used as a topical bath to treat skin diseases.

*Croton lobatus* L. has recently been reported by traditional medicine practitioner in Nigeria as having a good therapeutic index on chronic wounds by applying its fresh leaves or dried powder on wound surface while the leaf-decoction is used to aid development of fetus. Hence, it is necessary to screen for the phytoconstituents of *C. lobatus* and evaluate its antimicrobial activities against common microorganisms that infect wounds.

### Methods and materials

#### Sample Collection

*Croton lobatus* L. was collected from Obokun Local Government Area of Osun state Nigeria and was confirmed in the Botanical Garden, Biological Science department of Ahmadu Bello University, Zaria, with voucher specimen number of 913.

#### Preparation of crude extract

Leaf of *C. lobatus* was harvested and air dried at normal temperature. The dried powder of the plant was extracted with distilled for 24 h. The extract was filtered through Whatmann filter paper No 1 and it was thereafter, centrifuged at 5000G for 15 min. The supernatant were further filtered through Millipore filter before storage at 4°C as described by (Prashanthi et al., 2012).

#### Preliminary phytochemical test

Screening for the phyto-constituents such as saponins, alkaloids, flavonoids, steroids, tannins, cardiac glycosides, glycosides, and proteins was carried out as described by (Evans and Trease, 1996; Harborne, 1973; Sofowara, 1993).

### Table I. Concentration of *C. lobatus* water extracts and standard antibiotics used for both in vitro and in vivo antimicrobial studies

| Antibiotic disk cartridges (Treatment) | Code   | Disk Potency (treatment dose)     |
|--------------------------------------|--------|---------------------------------|
| 1  C. lobatus                         | CL – P (A) | Dried Paste (Liberal)        |
| 2  C. lobatus                         | CL – 0.5 (B) | 0.5 g/mL of water (2 drops)  |
| 3  C. lobatus                         | CL – 0.05 | 0.05 g/mL of water            |
| 4  C. lobatus                         | CL – 0.005 | 0.005 g/mL of water           |
| 5  Ceftriaxone                        | CRO 30  | 30 µg                           |
| 6  Erythromycin                       | E 15   | 15 µg                           |
| 7  Sulphamethoxazole                  | STX 25 | 25 µg                           |
| 8  Cloxacillin                        | OB 5   | 5 µg                            |
| 9  Oxacillin                          | OX 1   | 1 µg                            |
| 10 Ceftazidime                        | CAZ 10 | 10 µg                           |
| 11 Gentamycin                         | CN 10  | 10 µg                           |
| 12 Amoxycillin                       | AML 25 | 25 µg                           |
| 15 Physiological saline solution (PSS)| C     | 2 drops                          |
| 16 Cicastrin powder                   | D      | Liberal                          |

KEY: CL – P (C. lobatus Paste), CL – 0.5 (C. lobatus: 0.5 g/mL of water), CL – 0.05 (C. lobatus: 0.05 g/mL of water), CL – 0.005 (C. lobatus: 0.005 g/mL of water), CRO 30 (Ceftriaxone), E 15 (Erythromycin), STX 25 (Sulphamethoxazole), OB 5 (Cloxacillin), OX 1 (Oxacillin), CAZ 10 (Ceftazidime), CN 10 (Gentamycin), AML 25 (Amoxycillin).
Quantitative analysis of the phytochemicals

Flavonoids, alkaloid saponin and tannin were determined as describes by Bohm and Kocipai-Abyazan (1994), Harborne (1973), Obadoni and Ochuko (2001) and Van-Burden and Robinson (1981) respectively. While the spectrophotometric method was used to estimate total phenol contents of the plant.

Examinations of Antimicrobial activity of the C. lobatus

Microbial isolate used were Staphylococcus aureus (ATCC 33591); Streptococcus Spp (By Dr Raji bacteria zoonosis laboratory, A.B.U); Pseudomonas aeruginosa (ATCC 9028); Proteus vulgaris (isolate identified from bacteria zoonosis laboratory VPH, A.B.U); Escherichia coli (ATCC 43895); Salmonella Spp (ATCC 4932).

In vitro antimicrobial assay

A method by Irobi et al. (1994) and Shinwari et al. (2009) was adopted. The prepared C. lobatus water extract was reconstituted with sterile distilled water similar to what was done by Ongsakul et al. (2009) to obtain a concentration of 0.5g/mL. Lower concentrations of 0.05 g/mL and 0.00 5g/mL of water were then prepared from the concentration of 0.5g/mL (Table I). Broth cultures with turbidity equivalent of 0.5 McFarland standards of known bacteria strains as listed earlier were prepared. Using a sterile cotton swab stick, the Mueller Hinton Agar (MHA) Plates were inoculated. Sterile cork borer was used to create wells of 5 mm diameter in the medium and 100 µl of C. lobatus 0.5g/mL, 0.05 g/mL and 0.005 g/mL extracts were filled into each of the wells. The plates were incubated at 37°C for 24 hrs. The diameters of the growth inhibition formed in mm were recorded. The zone of inhibition was compared with standard antibiotics (Table I).

In vivo antimicrobial assay

A male albino rat of 22 weeks old, weighing about 200g was selected from an inbred colony obtained from Jos, Plateau State. It was housed in a steal cage under normal temperature, humidity and light. The rat was fed with commercial rat feed (prepared from vital feed grower mash) and adequate water was provided. Ethical permission was granted by the Ahmadu Bello University Committee on Animal Use and Care. The permission number was ABUCAUC/2016/027. The animal was allowed to acclimatize for two weeks before the experiment. It was anesthetized by intra muscular injection of Rompun® (xyllazine hydrochloride) at the dosage of 5 mg/kg and ketamine hydrochloride at the dosage of 40 mg/kg. The whole body of the animal was cleaned with diluted Purit®. Its dorsal region was surgically prepared for aseptic surgery and indelible marker was used to make a sterile square shape (0.5 by 0.5 centimeter) in four different places (Fig. 1). Full-thickness skin incision was carried out to create the wounds. The wounds were left for 48 hrs to mimic the clinical condition, after which they were accessed and samples were collected for culture, identification and CFU. Respective treatment using C. lobatus (paste), C. lobatus (water extract), Physiological saline solution (PSS), Cicatrin powder was carried out after 48 hrs of wound creation (Table I) and samples were collected at day one, three, five and seven after initiation of treatments for CFU counts in nutrient agar and MacConkey agar.

Data analysis

The results were presented inform of tables and charts, with bars representing standard error of means.

Results and discussion

Phytochemical Studies

The qualitative studies on the leaves of C. lobatus water extracts revealed that it contains carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids, alkaloids and tannins (Table II). The percentage composition of the alkaloids, flavonoids, saponins, phenols and tannins in the leaves of C. lobatus water extract is presented in the Table II. This study revealed that C. lobatus contains carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and...
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Table II. Phytochemical constituents of *C. lobatus*

| S/N | Chemical Constituents/ Test reagent | Qualitative test | Quantitative test (%) |
|-----|----------------------------------|-----------------|-----------------------|
| 1   | Carbohydrates                    |                 |                       |
|     | Molisch’s test                   | +               |                       |
|     | Fehling’s test                   | +               |                       |
| 2   | Cardiac glycoside                |                 |                       |
|     | Kella-killiani test              | +               |                       |
|     | Kedde’s test                     | +               |                       |
| 3   | Free Anthraquinones              |                 |                       |
|     | Borntrager’s test                | -               |                       |
| 4   | Combined Anthracene              |                 |                       |
|     | Modified Borntrager’s test       | -               |                       |
| 5   | Saponins                         |                 | 3.04                  |
|     | Frothing test                    | +               |                       |
|     | Hemolysis test                   | +               |                       |
| 6   | Steroids and Triterpenes         |                 |                       |
|     | Leiberman-Burchards test         | +               |                       |
| 7   | Flavonoids                       |                 | 8.60                  |
|     | Shinoda test                     | +               |                       |
|     | Sodium hydroxide test            | +               |                       |
| 8   | Tannins                          |                 | 3.80                  |
|     | Lead sub-acetate test            | +               |                       |
|     | Ferric chloride test             | +               |                       |
| 9   | Alkaloids                        |                 | 5.22                  |
|     | Mayer’s test                     | +               |                       |
|     | Dragendorff’s test               | +               |                       |
| 10  | Phenols                          |                 | 3.40                  |

Key: +: Present, -: Absent

Fig. 2. The clear zone of inhibitions (mm) of various concentration of *C. lobatus* plant extract compared with standard antibiotic drugs

**Antibacterial activities**

*Croton lobatus* L. showed a dose dependent activity against microorganisms. *Croton lobatus* paste and *C. lobatus* 0.5 retarded the growth of *Staphylococcus aureus*: *Streptococcus Spp*: *Pseudomonas aeruginosa*: *Proteus vulgaris*: *E. coli* and *Salmonella Spp* at the zone of inhibition ≤ 21mm. The *C. lobatus* 0.05 inhibited the growth of *Proteus vulgaris* at 34mm while *C. lobatus* 0.005 inhibited the growth of *Staphylococcus aureus* at 18mm (Fig. 2). CFU counted on nutrient agar reduced drastically after initiation of treatment (day 2) in all the treated wounds (A, B and D) except with physiological normal saline treated wounds (Fig. 3). On the MacConkey agar, CFU counted significantly reduced with days on cicafrin treated wounds, slightly reduces with days on the wounds treated with *C. lobatus* paste while persistent high number of CFU tannins as contained in other species of *Croton* (Attioua, 2005; Burkill, 1985; Chabert et al., 2006; Farnsworth, 1969; Willaman and Li, 1970). *Croton lobatus* L. contained 5.22 % alkaloid that was classified into five different types by Barthlemy et al. (2012). The study also revealed that *C. lobatus* contains steroids and triterpenes, flavonoids, alkaloids, and saponin similar to other species of *Croton*. Similar studies have revealed the presence of triterpenes (Babosa et al., 2003), steroids, flavonoids (Cai et al., 1991; Salatino et al., 2007) alkaloids (Aboagye et al., 2000; Amaral and Barnes, 1998; Milanowski et al., 2002; Yibrilign, 2007) triterpenoids, either pentacyclic or steroidal (Nath et al. 2013) and saponin (PROTA, 2011).
count was recorded with physiological normal saline treated wounds and C. lobatus water extract (Fig. 4).

The in vitro antimicrobial assay of C. lobatus plant extracts showed a dose depended activity against the tested microorganisms while the in vivo antimicrobial studies reveals that C. lobatus is very active against organisms that grow best in nutrient agar like Bacillus spp, Streptococcus spp, E. coli, Pseudomonas and Staphylococcus spp. Hence, it is active against microbial wound infections thereby preventing delayed wound healing. The antimicrobial property can be attributed to tannins (Christian et al., 2016;...
Fakhim et al., 2015), triterpenoids and flavonoids (Akpal et al., 2015; Thakur et al., 2011) crotonic acids (Goldstein et al., 2003; Michalik and Wahl, 2007) and saponin (Thakur et al., 2011) that is present in the leaves. This finding is supported by several other scientists who reported the antimicrobial activities of other species of croton (Abo et al., 1999; Dadson et al., 2012; McChesney et al., 1991; Peres et al., 1997; Salatino et al., 2007).

Conclusion

This study has revealed that C. lobatus contains secondary metabolites such as carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and tannins, which are responsible for its antimicrobial property. Hence, this explains its usage by the local medicine practitioner in Nigeria and various part of the world as alternative therapy in the management of diseases whose symptoms involve microbial infections. Further research is required on this plant.

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the phytoconstituents of aid development of fetus. Hence, it is necessary to screen for powder on wound surface while the leaf-decoction is used to index on chronic wounds by applying its fresh leaves or dried (Burkill, 1985). The plant is used generally as healing leaf in Africa, is commonly used by Indigenous people...

Methods and materials
Rompun (R) (xylazine hydrochloride) at the dosage of 5 mg/kg was allowed to acclimatize for two weeks before the permission number was ABUCAUC/2016/027. The animal growth inhibition formed in mm were recorded. The zone of 0.005 g/mL extracts were filled into each of the wells. The antibacterial activities of water were then prepared from the concentration of

Results and discussion
The results were presented inform of tables and charts, with counts significantly reduced with days on cicatrin treated wounds (Fig. 3). On the MacConkey agar, CFU of Salmonella Spp; C. lobatus; Salmonella Spp; E. coli; C. lobatus; and E. coli; C. lobatus; contained 5.22 %; McChesney et al., 2000; Peres 2003; Michalik and Wahli, 2007) and saponin (Thakur 2012). The study also revealed that Staphylococcus spp.

Antibacterial activities
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