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# Original article

# Phytochemical evaluation, antioxidant and COX-1 / COX-2 inhibitory activities of the methanol extract of the leaf of *Cochlospermum planchonii* Hook. F. (Cochlospermaceae)

**Paul Ngbede Olotu[1], Abubakar Ahmed[2], Oluyemisi Folashade Kunle[3], Ijeoma Adanma Olotu[4], Francis Maji Agwom[5], Ukpe Ajima[5]**

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences; [4]Department of Biochemistry, Faculty of Medical Sciences; [5]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences; University of Jos, Jos-930001, Plateau State, Nigeria.
[2]Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.
[3]Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Garki, Abuja, Nigeria.

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## Corresponding author

**Paul Ngbede Olotu**

Lecturer,
Department of Pharmacognosy and Traditional Medicine,
Faculty of Pharmaceutical Sciences,
University of Jos, PMB 2084,
Jos-930001, Plateau State, Nigeria.
Phone: +234-8035939772
Email: olotupauln@yahoo.com

## Abstract

*Cochlospermum planchonii* is widely used in folk medicine in many African countries and has been shown to have beneficial activity in a number of disease conditions. The plant is proposed to contain anti-inflammatory compounds that can relieve pain and inflammation associated with elevated levels of prostaglandins in the body. The present study was therefore aimed at investigating the phytochemicals present in the methanolic extract of the leaves of *Cochlospermum planchonii* L. (Cochlospermaceae) and also evaluating the antioxidant and COX-1/COX-2 inhibitory activities of the extract. Antioxidant activity was determined using a 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Phytochemical evaluation performed using standard known methods revealed the presence of steroids/terpenes, cardiac glycosides, saponins, tannins, flavonoids and alkaloids. The extract had significant antioxidant activity of 93±0.11% in the DPPH assay. The extract was found to inhibit COX-2 selectively. The preliminary antioxidant screening indicated that the extract possesses the potential to scavenge free radicals and the capacity to bleach DPPH. The COX assay was carried out with a view to providing a simple mechanism by which the extract exhibits its anti-inflammatory action. The present results are consistent with the traditional use of this plant in phytotherapeutic preparations for managing pain and inflammation related ailments.

**Key words:** Cyclo-oxygenase inhibition, DPPH, Ethnopharmacology, Free radicals, Secondary metabolites

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*Cochlospermum planchonii* is a low shrubby savanna plant that can grow as high as 2.5 meters and reproduces from the seeds and rhizomes. Among the tribes of Nigeria, it is called "N′ Dribala" (Fulani), "Rawaya" or "Kyamba" (Hausa), "Abanzi" (Igbo) and "Gbehutu or Feru" (Yoruba). The plant is commonly used as a traditional medicine in several sub-Saharan African countries such as Cote d'Ivoire, Nigeria, Burkina Faso, Ghana, Cameroon, Guinea, Gambia and Senegal[1]. It is also a common weed of cultivated fields in the Sudan and Guinea savannah zones[2]. The leaf, stem and root bark of *C. planchonii* have all demonstrated strong antifungal activity against *Colletotrichum capsici*[3]. The aqueous leaf extract of the plant has been shown to have blood glucose lowering and antidyslipidemic potential[4] while the essential oil of the plant has been shown to possess antibacterial activity[5]. The decoction of the plant leaves has also been used as an alternative therapy for diarrhoea[6], gonorrhoea, jaundice and gastrointestinal disease[7]. Analysis of the root extract showed the presence of egallic acid, zinc salt and manganese[8].

Reactive oxygen species (ROS) or sometimes reactive nitrogen species (RNS) are highly reactive entities containing an unpaired electron. Some prominent examples of these species include superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), peroxyl radical (HO), singlet oxygen ($^1O_2$) and peroxynitrite ($ONOO^-$). They are known to cause damage to human cells[9]. To neutralize the assault of these ROS, living cells have a biological defense mechanism made up of enzymatic antioxidants that convert reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as $O_2\bullet^-$ and NO• to harmless species. However, no enzymatic action is known to scavenge radicals such as $ROO^{\bullet}$, $^{\bullet}OH$, $^1O_2$, and $ONOO^-$. Therefore, the burden of defense relies on a variety of non-enzymatic antioxidants such as vitamin C and E and many phytochemicals that have the property of scavenging oxidants and free radicals[10]. An antioxidant is any substance that, when present at low concentrations, compared with those of an oxidizable substrate (lipids or proteins, etc.) significantly delays or prevents oxidation of that substrate. It can be broadly classified as enzymatic such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) and their precursors or non-enzymatic such as vitamins from fruits, polyphenols from vegetables and medicinal plants. Antioxidant protection may be based on several mechanisms of action, which include: scavenging capacity against ROS/RNS, reducing capacity, metal chelating capacity, anti-oxidative enzymes e.g. nitric oxide synthase, xanthine oxidase and cyclooxygenase[11]. A number of phytochemicals obtained from plants have been shown to possess antioxidant activity and can serve as lead compounds for the development of new drugs[12].

Inflammation is a response that occurs in living tissues to injury, infection or irritation and is characterized by pain, swelling, redness and heat. Inflammatory abnormalities are a large group of disorders that underlie a wide variety of human diseases such as hay fever, cancer, ischemic heart disease, rheumatoid arthritis, etc[13]. The term eicosanoid covers biologically active lipid mediators ($C_{20}$ fatty acids and their metabolites) including prostaglandins, thromboxanes, leukotrienes and other oxygenated derivatives, which are primarily produced by three classes of enzymes; cyclooxygenases (COX), lipoxygenases (LOX) and cytochrome P450 mono-oxygenases. The immediate precursor of the eicosanoids is arachidonic acid which is hydrolyzed from phospholipids by Phospholipids A2, also a membrane component[14]. Eicosanoids are implicated in pains, inflammatory conditions[15]. Cyclooxygenases (COX) is an enzyme that catalyzes the conversion of arachidonic acid (AA) into a large number of eicosanoids with potent biological effects[14].

*Cochlospermum planchonii* L. (Cochlospermaceae) leaf is locally used as medicine without any scientific validation. The chemistry of Cochlospermaceae is poorly known[16]. In the present study, the methanolic extracts of the leaves of *Cochlospermum planchonii* was therefore chosen to validate its traditional use in folk medicine.

## Materials and methods

### *Plant collection, identification and preparation*

*Cochlospermum planchonii* leaf was collected on the 9th June 2012 from 'Babare' locality, in Jos North Local Government Area of Plateau state, Nigeria. The plant was identified in the field using the pharmacognostic descriptions and keys. The identity of the plant was authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Nigeria, and assigned Voucher specimen Number (FHJ 1011). The plant was collected and air dried at room temperature under shade until a constant weight was obtained for a period of three weeks. The plant was then pounded to powder using a pestle and mortar, sieved with a mesh of size-20 and stored in an air-tight container until when required for use.

*Chemicals and reagents*

All the solvents used in the study were of analytical grade.

*Equipment*

UV-Visible double beam spectrophotometer Model UV-1620PC (Shimadzu, Japan), Beckman Model LS 6000LL Liquid Scintillation Counter (Beckman Instruments, Palo Alto, United States of America), Rotary Evaporator Rotavapor R-300 (BUCHI, Switzerland).

*Plant extraction*

The powdered drug (1 kg) was extracted with methanol (3 Liters) by maceration/electric shaking for 72 hours. The extract was concentrated under reduced pressure using a Rotary evaporator to obtain the methanol extract (18.40% yield).

*Phytochemical screening*

The presences of phytochemical constituents of the different extracts were investigated using standard methods[17].

*Thin Layer Chromatographic (TLC) analysis*

The TLC profile of the methanol extract was obtained by spotting the extract on the TLC plate and developing it in Ethyl acetate: Methanol: Hexane (7:7:3). The plates were sprayed with anisaldehyde / sulphuric acid as detecting reagent and heated at 110°C. The chromatogram was scanned using a previously described method[12].

*Antioxidant assay*

It was carried out on pre-coated silica thin layer chromatography (TLC) plate and appropriate solvent systems. The 2, 2-Diphenyl-l-Picrylhydraxyl (DPPH) reagent was used as detecting reagent. A yellow spot against purple background would suggest presence of free radical scavenging compounds. The antioxidant activity (free radical scavenging activity) of aqueous methanolic extract on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method described by (Brand-Williams et al, 1995). 12.5 mg of the extract was dissolved in methanol using 25 mL volumetric flask. The following concentrations of aqueous methanolic extract were prepared 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95, 0.98, 0.49, 0.245, 0.1225; 0.06125 µg/mL. All the solutions were prepared with methanol as solvent. About 2 mL of each prepared concentration was mixed with 4 mL of 50 µM DPPH solution in methanol. The experiment was done in triplicate. The mixture was vortexed for 10 seconds to homogenize the mixture and test tubes were incubated for 30 mins at room temperature in the dark. After 30 minutes of incubation, the absorbance was measured at 515 nm on a UV-Vis spectrophotometer**.**

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Gallic acid, vitamin C and rutin were used as standards in the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195 µM. Blank solution was prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH solutions. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\%\ Inhibition = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\ X\ 100 \ldots\ldots equation\ 1$$

Finally, the $IC_{50}$ value, defined as the concentration of the sample leading to 50 % reduction of the initial DPPH concentration, was calculated from the separate linear regression of plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL).

*Prostaglandin synthesis (COX) inhibition assay*

Anti-inflammatory activity was determined using the COX-1 and COX-2 inhibitory assays as described[18,19]. Indomethacin, a known COX-1 and COX-2 inhibitor was used as control. A stock solution of COX-1 enzyme (60 $\mu L$) stored at -70 °C was activated with 1250 $\mu L$ of co-factor solution (0.3 mg/ml Epinephrine, 0.3 mg/ml reduced glutathione in 10 ml of Tris buffer pH 8.0, and 100 $\mu$L hematin solution) and pre-incubated on ice for 5 mins. Samples were first screened at 10 mg/mL (crude extracts) and 1 mg/ml (compounds). In duplicate, plant extract (2.5 $\mu L$) was added to 17.5 $\mu L$ of distilled filtered water in Eppendorf tubes to give a final concentration of 250 $\mu g$/m*L* of organic extract per test solution. A similar preparation of 5 $\mu$M indomethacin was used as a positive control. Solvent blank and background (2.5$\mu L$ ethanol + 17.5 $\mu L$ of water) were used as negative controls. The enzyme solution (60$\mu L$) was added to the test solution and pre-incubated at room temperature for 5 mins. The reaction was started by adding 20 $\mu L$ of $^{14}$C-arachidonic acid to the test solutions, with the enzymes in the background being inactivated by adding 10 $\mu L$ 2N hydrochloric acid (HCl) be-

fore incubating the test solution at 37 °C for 10 mins.

The incubation was stopped and the reaction terminated by adding 10 $\mu L$ 2N HCl to each test solution. Unlabeled prostaglandins (4 $\mu L$ of 0.2 mg/mL) as a carrier solution were then added to each test solution. The samples were loaded onto individual Pasteur pipettes packed with silica gel (particle size 0.063-0.200 mm, Merck) with 1 mL of hexane: 1, 4**-** dioxan: acetic acid (70:30:0.2 v/v/v) to separate prostaglandins from the unmetabolized arachidonic acid.

Column chromatography was used to elute the unmetabolized arachidonic acid with 4 mL of hexane: l, 4-dioxan:acetic acid (1 mL at a time) while the elution and collection of prostaglandin products was done with 3 mL of ethyl acetate: methanol (85:15 v/v) into scintillation vials**.** Four mL of scintillation fluid was added to the eluate in the scintillation vials and the radioactivity was measured using a Beckman LS 6000LL scintillation counter. Percentage inhibition of the screened extracts was calculated using:

$$\% \, COX \; Inhibition = \left\{1 - \frac{DPMsample - DPMbackground}{DPMcontrol - DPMbackground} X\,100 \ldots\ldots \; equation\; 2\right.$$

DPM = Disintegrations per minute

## Results

**Table 1:** Result of the phytochemical analysis of the methanol extract of *Cochlospermum planchonii* leaf

| Test Reagent | Observation | Inference |
|---|---|---|
| Unsaturated Steroids/triterpenes (Liebermann-Buchard's test) | Upper layer: blue-green colour, lower layer: reddish colour | Present |
| Cardiac glycosides (Keller-Kiliani test) | Pale green colour at the interface (deoxy sugar) | Present |
| Saponins (Frothing/ Heamolysis Test) | Honey comb froth/heamolysis of RBCs | Present |
| Flavonoids (Shinoda Test) | Yellow colour | Present |
| Tannins ($FeCl_3$) | Greenish-Black ppt. | Present |
| Alkaloids (Dragendorff's and Mayer's Test) | 1. Cream ppt<br>2. Reddish brown ppt | Present |

ppt = precipitate, RBC = red blood cells

**Table 2:** Result of the thin layer chromatography of the methanol extract of *Cochlospermum planchonii* leaf

| Spot Position (cm) | $R_f$-value (cm) | Daylight | UV-254 (nm) | UV-366 (nm) | Anisaldehyde spray |
|---|---|---|---|---|---|
| 0.9 | 0.13 | Yellow | Yellow | Greenish | Greenish |
| 1.7 | 0.24 | Yellow | Yellow | Greenish | Greenish |
| 2.8 | 0.40 | Light Green | Light Green | Greenish Blue | Greenish Blue |
| 4.2 | 0.60 | Brown | Brown | Brown | Brown |
| 5.1 | 0.72 | Yellow | Yellow | Brown | Brown |
| 5.4 | 0.77 | Light Green | Light Green | Greenish Blue | Greenish Blue |
| 6.1 | 0.84 | Brown | Brown | Greenish Blue | Greenish Blue |

**Table 3:** Result of the effect of the methanol extract of *Cochlospermum planchonii* leaf on prostaglandin synthesis

| Sample | Prostaglandin Synthesis (%) | |
|---|---|---|
| | COX-1 | COX-2 |
| Methanol extract | 45.4 ± 6.2 | - |
| Indomethacin | 46.3 ± 3.2 | - |

## Discussion

Inflammation is a complex immune response in living organisms that helps to defend them against harmful stimuli such as mechanical injury, pathogens, or irritants[20]. Initiation of the inflammatory process leads to release of a number of mediators and persistence of low-grade inflammation has been linked to the pathogenesis of various disorders, including cancer, atherosclerosis, rheumatoid arthritis, type-2 diabetes, and vascular diseases[20,21]. Quite a number of steroidal and non-steroidal anti-inflammatory drugs (NSAID's) are presently being used in the treatment of inflammatory disorders. They are however associated with numerous side effects such as cardiovascular, renal failure and gastrointestinal bleeding[22]. Researchers are therefore putting a lot of effort into the development of new anti-inflammatory agents from natural sources that will hopefully have fewer adverse effects than the synthetic drugs[23-25]. In this study, the methanol leaf extract of *Cochlospermum planchonii* was evaluated on its ability to inhibit COX-1 and COX-2 enzymes which are involved in the biosynthesis of prostaglandins. The COX assay (Table 3) was carried out with the view to ascertaining the mechanism by which the methanol extract exhibits its anti-inflammatory action. The extract was found to inhibit COX-2 selectively at (45.4 ± 6.2%). This is hugely beneficial as COX-2 selective inhibitors have the advantage of lower risk of gastrointestinal bleeding when compared to non-selective inhibitors. The result is also in agreement with a previous study[26] which used the Carregenan induced paw edema method in rats to establish the anti-inflammatory activity of the methanolic root extract of the plant.

The phytochemical constituents of the methanolic leaf extract were investigated and it revealed the presence of phytochemicals such as steroids/terpenes, cardiac glycosides, saponins, tannins, flavonoids and alkaloids (Table 1). An earlier report had also found that the methanolic root extract of the plant contained similar phytochemicals such as flavonoids, glycosides and tannins[26]. Some of these classes of phytochemicals have been linked with a number of activities. For example, Saponnin-rich plants have been shown to demonstrate anti-inflammatory and anticancer properties[27] while polyphenolics have been shown to have antioxidant activity among others[28]. The chromatogram of the extract showed abundant greenish spots when viewed under UV lights of 254 and 366 nm. This can be attributed to the presence of phenolic compounds[29]. Furthermore, Flavonoids are known to have potent anti-oxidant and anti-inflammatory properties. They have the ability to scavenge free radicals such as hydroxyl and lipid peroxy radicals[30,31] which have been implicated in causing some diseases such as diabetes, Parkinson and Alzheimer's diseases. The presence of these phytochemicals in the extract may therefore account for its traditional use in treatment of some diseases.

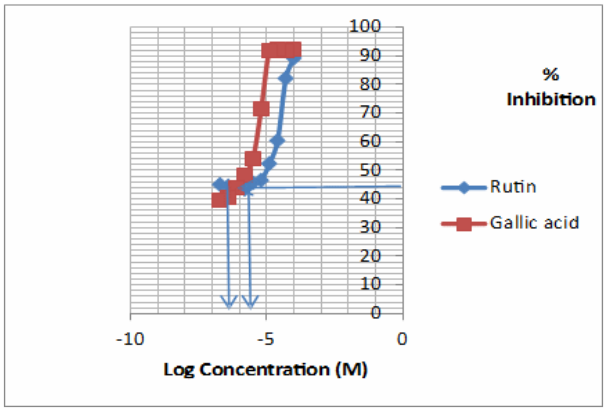


**Fig 1.** Free radical scavenging activity of Rutin & Gallic acid

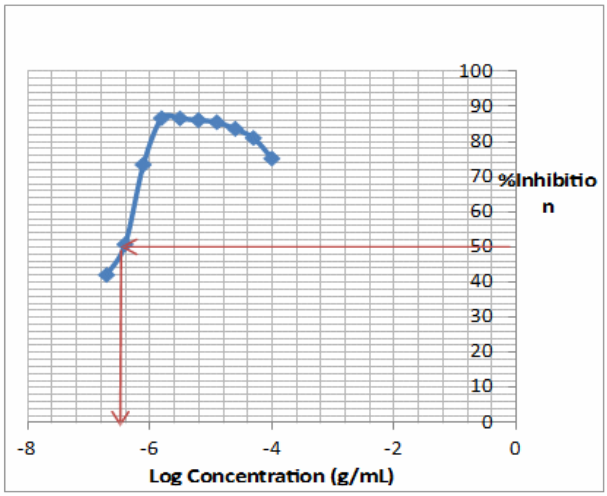


**Fig 2.** Free radical scavenging activity of Vitamin C

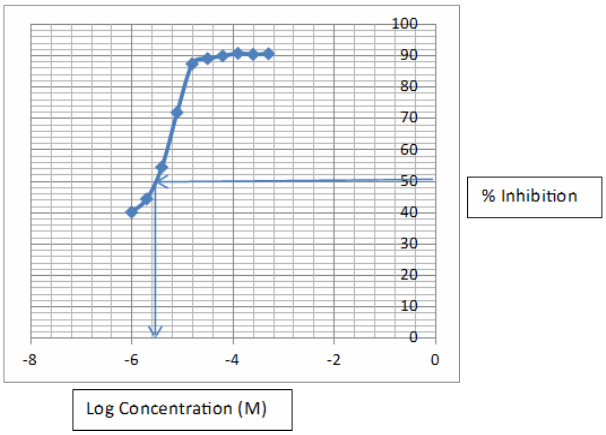


**Fig 3.** Free radical scavenging activity of methanol leaf extract

The antioxidant activity of the extract was determined using the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay and employing ascorbic acid as standard control (Fig 2). The extract had significant antioxidant activity of 93 ± 0.11% in the DPPH assay as compared with ascorbic acid, rutin and gallic acid which had antioxidant activity of 73 ± 0.12%, 90 ± 0.13%., 73 ± 0.15 % respectively. The preliminary antioxidant screening indicated that the extract possesses the potential to scavenge free radicals and the capacity to bleach DPPH even better than all the controls. This can, as well, be attributed to the presence of phenolic compounds[31].

## Conclusion

The methanol leaf extract of *Cochlospermum planchonii* has a selective COX-2 inhibitory activity against prostaglandin synthesis due to the presence of phenolic compounds which are also responsible for the antioxidant activity. Additional work is currently on going in our laboratory in an effort to isolate, characterize and identify these specific compounds for further drug development.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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