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Research Article

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Determination of Total Phenolic/Flavonoid Content and Analgesic Properties of Different Extracts of *Cochlospermum planchonii*, Hook. F (Cochlospermaceae)

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Abstract Medicinal plants contain substances that are used for therapeutic purposes or as precursors for synthesis of useful drugs. Since time past, they have been used in virtually all cultures as a source of medicine. The present study is to determine the total phenolic/flavonoid contents and analgesic properties of different leaf extracts of *Cochlospermum planchonii* Hook. F (Cochlospermaceae) using acetic acid induced writhings in both sexes of Swiss albino mice. From the results, the total phenolic content of the hexane, ethyl acetate and methanol extracts calculated from the calibration curve (r2=0.997, 0.998, 0.999) were 50.45±1.66, 47.33±15.23 and 239.11±36.60 Gallic Acid Equivalents/g respectively, while the total flavonoid content (r2=0.992, 0.993, 0.997) were 4.94±0.50, 23.60±0.16 and 26.75±0.41 Rutin Equivalents/g respectively. The quantitative values of these secondary metabolites are quite appreciable and are used as the basis for screening the drug for antioxidant activity. The analgesic activity associated with the different leaf extracts for n-hexane, ethyl acetate and methanol extracts were also determined. From the results, the different extracts showed a significant difference ($p < 0.05$) in the percentage inhibition of the acetic acid induced writhing in mice in a dose related pattern with the methanol extract giving the highest activity. This extract is rich in phenolic compounds which are known to exhibit analgesic effects in animal models. It can therefore be concluded that the analgesic activity claimed by the traditional medical practitioners may have a scientific basis.

Keywords Antioxidant, Analgesic, Medicinal Plants, *Cochlospermum planchonii*, Phenolic

Introduction

Medicinal plants are plants which contain substances which could be used for therapeutic purposes or which contain chemicals that could serve as precursors for the synthesis of useful drugs [1]. Medicinal plants have been used since ancient times by virtually all cultures and civilizations as a source of medicine. The widespread benefits of plants and other healthcare formulations as those stated in the ancient and holy texts such as Vedas and the bible have also

been traced to the occurrence of natural products with medicinal properties. This use has been widely observed in most developing countries, including Nigeria [2].

Medicinal plants typically contain active principles that may act either individually, additively or synergistically to improve health. A single plant may for example be used as anti-inflammatory, anti-oxidant, anti-bacterial and anti-fungal agents [3]. Several reasons have been documented on why people use medicinal plants as medicine. Firstly, they believe that medicinal plants are more effective than the modern medicine and there is no record of any side effect. Secondly, it is also believed that drug-drug interactions takes place in the earthen pot used for the preparations and not in the stomach. Furthermore, traditional medicine is said to be compatible with the human system as both are living entities and so not antagonize each other. There is also a belief that traditional medical practices form parts of the norms, belief and culture of the people and therefore, must be wholly accepted since it can also be used to word out evil spirits [4, 5].

The capacity of phenolic compounds especially flavonoids to act as antioxidants *in vitro* has been the subject of several studies in the past years [6] and the structural requirement considered to be essential for effective radical scavenging by phenolic compounds such as flavonoids has also been established [7]. Flavonoids and saponins are known to exhibit analgesic effects on animals [8].

Cochlospermum planchonii Hook. F (Cochlospermaceae) leaf has been used traditionally as an analgesic and anti-inflammatory agent but so far, no scientific work was done on the leaf to justify the claim [9]. The present study is therefore aimed at the determination of the total phenolic/flavonoid content and the analgesic study of the different leaf extract of *Cochlospermum planchonii* with the view to propose a simple mechanism for the analgesic properties.

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 identity was authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Nigeria, and assigned Voucher specimen Number (FHJ 1010). The plant was air dried at room temperature under shade for a period of three weeks. The plant was then pounded to powder using local 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.

Plant Extraction

The drug powder (1kg) was sequentially extracted with hexane (3L), ethyl acetate (3L) and methanol (3L) by maceration/electric shaking for 72 hours in each case. The extracts were concentrated under reduced pressure using Rota-vapor to have Hexane extract (2.76 % yield), Ethyl acetate extract (15.57 % yield) and Methanol extract (18.40 % yield) [10].

Determination of Total Phenolic Content

Total phenolic contents of the extract were evaluated with Folin-Ciocalteu method according to a previously described method [11]. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent thereby producing blue coloured complex. The phenolic concentration of extract was evaluated from a gallic acid calibration curve. 500 µL aliquots of 10, 20, 30, 40, 50, and 60 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The tubes were vortexed for 10 seconds and allowed to stand for 30 minutes at 25^{o}C. After incubation at 25°C for 30 minutes, absorbance was measured at 765 nm against reagent blank using the UV-Vis Spectrophotometer 1650 Shimadzu, Japan. Total phenolic content was expressed as mg/g gallic acid equivalent using the following equation based on the calibration curve: $y = 0.0069 x + 0.0673$, $r^2 = 0.9947$, where x was the absorbance and y was the gallic acid equivalent (mg/g).

A similar procedure was adopted for the extracts as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

Determination of Total Flavonoid Content

Flavonoid quantification was done using Aluminum Chloride colorimetric method [12]. Plant extract (500 µL) was mixed with 1.5 ml of methanol, 100 µL of 1M sodium acetate and 2.8 mL of distilled water and kept to incubate at room temperature for 40 minutes. The absorbance of the reaction mixture was measured at 415 nm. All experiments were performed in triplicates and data were expressed in terms of flavonoid content (Rutin equivalent, RE) per g dry weight of the extracts. The calibration curve was prepared by using 13 to 500 µg/mL of Rutin in methanol (linear regression $r^2 = 0.9957$ with equation: $y = 0.0001x + 0.0674$).

Animals

Healthy Swiss albino male and female mice (25 to 30 g) obtained from the animal house of the Department of Pharmacology, University of Jos, Jos Nigeria were used for the study. The experimental animals were fed with standard pellet feed and water *ad-libitum* and kept in standard cages under laboratory condition. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals and the study protocol was approved by the Institutional Animal Care and Use Committee.

Acute toxicity studies

The method previously described [13, 14] with slight modifications were used for Acute Toxicity Studies. The study was conducted in two phases. In the first phase, three groups of four mice each were administered with the various extracts of *Cochlospermum planchonii* at the respective oral doses of 10, 100, and 1000 mg/kg body weight. The rats were observed for signs of toxicity such as any change in skin and fur, eyes and mucus membrane (nasal), breathing and changes like salivation, lacrimation, perspiration, piloerection, urinary incontinence ptosis, drowsiness, gait, tremors and convulsion and possible deaths for 24 hours, 72 hours, 2 weeks and then, four weeks. There was no death recorded in the first phase so, the experiment was preceded to the second phase. In the second phase, another three groups of four rats each were given respective doses of 1500, 2900 and 5000 mg/kg body weight of the extract and were also monitored as in phase one for toxicity signs and possible deaths. From the data obtained, LD_{50} was then calculated.

Acetic acid Induced Abdominal Writhing in Mice

The method described by [15] was used. Twenty five Swiss albino male and female mice (25 to 30 g) were shared into five groups of five mice each. Group I which served as the control group received distilled water 10 ml/kg, orally. Group II, III, and IV received extract dissolved in distilled water at the doses of 1500, 2000 and 2500 mg/kg intraperitoneally respectively while group V received acetylsalicylic acid 100 mg/kg dissolved in distilled water subcutaneously which were administered 30 minutes before intraperitoneal injection of 0.6 % v/v acetic acid solution in normal saline at a dose of 10 ml/kg. Immediately after administering acetic acid, mouse – pairs were placed in transparent glass cages and the number of writhings or stretches were counted for 15 minutes. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect. The data were computed according to the following formula:

$$\text{Percentage Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100\ \%$$

This was repeated for the three (3) different extracts (n-hexane, Ethyl acetate and Methanol extracts).

Statistical analysis

The results were expressed as mean ± Standard Deviation (SD) using one-way Analysis of variance (ANOVA). Differences were considered statistically significant at $p<0.05$.

Results

Table 1: Quantitative Values of Total Phenolic/Flavonoid Contents of the different Extracts of *Cochlospermum planchonii* Leaf

Extract	TPC (mg GAE/g) Extract	TFC (mg RE/g Extract)
Hexane	50.45 ± 1.66	4.94 ± 0.50
Ethyl acetate	47.33 ± 15.20	23.60 ± 0.19
Methanol	239.11 ± 36.60	26.75 ± 0.41

Key

TPC- total phenolic content, TFC- total flavonoid content, GAE- gallic acid equivalent, RE- rutin equivalent. The results are average of triplicate analysis (n = 3; data expressed as Mean ± SD).

Table 2: Effect of Hexane Extract of *Cochlospermum planchonii* Leaf on Acetic Acid Induced Writhings in Mice

Group	Treatment	Dose (mg/kg)	Writhing (M±SEM)/ 10 min	% Inhibition
1	N/S	-	50.55 ± 2.45	-
2	Extract	5	5.60 ± 2.45[a]	56
3	Extract	10	3.20 ± 0.58[a]	65
4	Extract	15	2.00 ± 0.32[a]	70
5	ASA	5	1.80 ± 0.37[a]	80

[a] = Significant difference at (P< 0.05) N=5

Table 3: Effect of Ethyl acetate Extract of *Cochlospermum planchonii* Leaf on Acetic Acid Induced Writhings in Mice

Group	Treatment	Dose (mg/kg)	Writhing (M±SEM)/ 10 min	% Inhibition
1	N/S	-	50.55 ± 2.45	-
2	Extract	5	4.40 ± 2.45[a]	60
3	Extract	10	3.50 ± 0.58[a]	70
4	Extract	15	2.30 ± 0.32[a]	75
5	ASA	5	1.80 ± 0.37[a]	80

[a]=Significant difference at (P< 0.05) N=5

Table 4: Effect of Methanol Extract of *Cochlospermum planchonii* Leaf on Acetic Acid Induced Writhings in Mice

Group	Treatment	Dose (mg/kg)	Writhing (M±SEM)/ 10 min	% Inhibition
1	N/S	-	50.55 ± 2.45	-
2	Extract	5	3.60 ± 2.45[a]	65
3	Extract	10	3.20 ± 0.58[a]	75
4	Extract	15	2.00 ± 0.32[a]	78
5	ASA	5	1.80 ± 0.37[a]	80

a = Significant difference at (P< 0.05) N=5

Discussion

Table 1 shows the result of the total phenolic content of the hexane, ethyl acetate and methanol extracts calculated from the calibration curve (r2= 0.997, 0.998, 0.999) were 50.45±1.66, 47.33±15.23 and 239.11±36.60 Gallic Acid Equivalents/g respectively, and the total flavonoid content (r2=0.992, 0.993, 0.997) were 4.94±0.50, 23.60±0.16 and 26.75±0.41 Rutin Equivalents/g respectively. From the result, the quantitative values of these secondary metabolites are quite appreciable and this could be used as the basis for screening the drug for antioxidant activity. Phenolic compounds have redox properties which allow them to act as antioxidant as their free radical scavenging ability is facilitated by their hydroxyl groups. Flavonoids also have antioxidant activity both *in vivo* and *in vitro* [16, 17].

The results of the analgesic studies of the different leaf extracts are depicted in (Tables 2, 3 and 4) for n-hexane, ethyl acetate and methanol extracts respectively. From the results, the different extracts showed a significant difference ($p< 0.05$) in the percentage inhibition of the acetic acid induced writhings in mice at dose related pattern with the methanol extract giving the highest activity. The acetic acid induced writhing test is commonly used as an experimental animal model for anti- nociception. The method is very sensitive and able to detect anti-nociceptive effects of compound (s) at dose level that may appear to be inactive in other methods like tail flick test [18]. The abdominal constriction response is postulated to partly involve local peritoneal receptors. Acetic acid is known to trigger the production of noxious substances such as prostonoids like PGE_2 and PGF_2 [19] as well as Lipoxygenase productions [20, 21] and cyclo-oxygenase etc. [22]. The analgesic effect exhibited by the extracts may be an indication that they depressed the production of the irritants and thereby reducing the number of writhes in the animals. The behavioural reaction (writhing) of the animals in this model is sensitive to drugs with activity similar to aspirin, an antagonist of kinin receptors and centrally/peripherally acting opioids analgesics [23, 24]. The analgesic effect of the extracts may be either due to its action on visceral receptor sensitive to acetic acid, to the inhibition of the algogenic substances or the inhibition of transmission of painful messages at the central level [25]. Flavonoids and saponins are known to exhibit analgesic effects on animals [8, 26, 27].

Conclusion

The different leaf extracts of *Cochlospermum planchonii* was demonstrated to contain phenolic compounds that was responsible for the analgesic activities claimed by the traditional medical practitioners.

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