ANTIOXIDANT EVALUATION OF ROOT EXTRACTS OF *Cochlospermum Planchonii* (KUNTH)

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Abstract: This work was aimed at evaluating the biological and anti-oxidant potential of the ethyl acetate and methanol fractions of the root of *Cochlospermum planchonii* and also to carryout biological activities on petroleum ether, Chloroform ethyl acetate and methanol. Chloroform gave the highest extractive value followed by methanol, petroleum ether and ethyl acetate. The antimicrobial screening of the root extracts of the plants were carried out on the following Microorganisms; Staphylococcus aureus, Escherichia Coli, Salmonella Typhi, Shigellia Dysenteric, Microsperum Canis, and Candida albicans. Klebselia pneumonia, Vibrio Cholerae and Trichophyton sp to determine their activities. The root extract showed that seven (7) of the microorganisms were sensitive to all the four extracts and two (2) were resistant to the extracts and the zone of inhibition observed for the microbes. The ethyl acetate extract show the highest zone of inhibition ranging from 24 to 28 mm and petroleum ether extract show the lowest values ranging from 16 to 18 mm. The antioxidant activities of the extracts have a high concentration which gives high percentage of DPPH inhibition with low inhibitory concentration (IC_{50}) value, of 1.06 µg/ml and 2.61 µg/ml for Methanol and ethyl acetate extracts respectively while the standard ascorbic acid have IC_{50} of 107.08 µg/ml as calculated from regression equation from the graph which means that the extracts were more active than the standard ascorbic acid in antioxidant properties as compared with standard ascorbic acid.

Keywords: Biological, anti-oxidant, *Cochlospermum planchonii*, Cochlospermum, IC_{50}, scavenging

Introduction

Medicinal plants are now more focused than ever, because they have the capability of producing many benefits to society indeed to mankind, especially in medicinal application. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body (Akinmoladun *et al*., 2007. The most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate healthcare service to rural people (Ghani 1990). Nature has long been an important source of medicinal agents. An impressive number of modern drugs have been isolated or derived from natural source, based on their use in traditional medicine.

Antioxidants are substances that have the ability to protect organisms from damage caused by free radical-induced oxidative stress. Both artificial and naturally occurring antioxidants have been reported to play major roles in protecting membranes and tissues from free radical oxidative damage (Burton, 1989; Carini *et al.*, 1990). It is very important property of medicinal plants because there are number of reports which mention that in biological systems, free radicals are causative agents for different disease such as cancer. Presently, the probable toxicity of synthetic antioxidants has been identified. It is strongly believed that regular consumption of plant-derived phytochemicals may drift the balance toward an adequate antioxidant status. A lot of research is being carried out worldwide directed toward finding natural antioxidants of plant origin. These form the bases in probing into the biological and antioxidant properties of *Cochlospermum planchonii* plant due to its use in traditional medicine.

Materials and Methods

Sample collection and preparation: The plant materials were collected from the bushes around Central Bank of Nigeria Zone 8, Lokoja, Kogi State, Nigeria in October 2015. It was authenticated with specimen voucher number 003 at the herbarium unit of the Department of Biological Sciences, Federal University Lokoja Nigeria. The plant material were air-dried for 21 days and crushed to coarse powder and kept away from moisture for analysis.

Extraction procedure: Two hundred grams (200 g) of the powdered root of the plant material was extracted by cool maceration with 500 ml petroleum ether, 400 ml chloroform, 300 ml ethyl acetate and 300 ml methanol successively. The resulting mixture was filter using Whatman No. 1 filter paper into round bottom flask after 24 h. The filtrate was poured into beakers and allowed to dry and obtain the crude extracts.

Biological activities: Diffusion method of Bauer *et al.* (1966) and Barry and Thornsberry (1985) were used in the determination of the biological activities of the various extracts.

Anti-oxidant analysis: The radical scavenging abilities of the *Cochlospermum planchonii* root extract on stable 1, 1-diphenyl-2-picrylhydrazyl (DDPH) radical were qualitatively and quantitatively determined.

Qualitative assay

Dilute solutions of the *Cochlospermum planchonii* root extracts were prepared with ethanol and spotted on pre-coated silica gel TLC plates (G254, Merck) with capillary tube. The TLC plates was developed in solvent systems of different polarities (polar-Methanol/Ethyl acetate; 1:2, medium polar-ethyl acetate/petroleum ether; 3:1 and non-polar- petroleum ether/ethyl acetate; 3:1) to resolve polar and non-polar component of the extracts. The plates were allowed to dry at room temperature and sprayed with 0.02% DPPH in methanol. Bleaching of DPPH by the resolved spots were observed for 10 minutes and after then Colour changes were observed for the spots (yellow on purple background) (Sadhu *et al.*, 2003).

Quantitative assay

Quantitative assay was done to determine the quantity of antioxidants (in percentage) in the *Cochlospermum planchonii* root extracts. Stock solution (1 and 0.8 mg/ml) of the *Cochlospermum planchonii* root extracts were prepared in methanol and serial dilutions were made to obtained for -100, -200, -400, -500 µg/ml solutions. Free radical scavenging activity of the extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Aldrich, USA) using Blois method (1958), modified by Saha *et al*. (2004). Dilute extract solutions (2 ml) were added to 2 ml of 0.1mM of methanol solution of DPPH. The mixture was mixed thoroughly and kept in the dark for 30 min. The absorbance was measured at 517 nm and from the values obtained, corresponding

percentage of inhibition were calculated. The percentage inhibition was plotted against log of concentration and from the graph IC_{50} was calculated. Ascorbic acid was used as a standard inhibitor while DPPH solution was used as control. All the tests were performed in triplicates and the graph was plotted with mean values. The percentage inhibition was calculated using the formula:

$$\% \text{ DPPH radical scavenging} = 1\text{-}A_s/A_c \times 100$$

Where, A_c= absorbance of control, and A_s= absorbance of sample solution.

Result and Discussion

Extraction: The weight and the percentage extract obtained from 200 g of the plant materials is as shown in Table 1. The Chloroform extracted more of the plant constituent followed by Methanol, Petroleum ether and Ethyl acetate, respectively.

Table 1: Amount of extract from 200 g of powdered root of *Cochlospermum planchonii*

Solvent Medium	Weight of extract (g)	Percentage of extract (%)
Petroleum ether	1.2	0.6
Chloroform	1.8	0.9
Ethyl acetate	0.7	0.35
Methanol	1.7	0.85

Biological screening: The biological activities was carried out on the following clinical isolates; *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholerae*, *Klebselia pneumonia*, *Salmonella typhi*, *Shigellia dysenteric*, *Microsperum canis*, *Trichophyton* sp and *Candida albicans*. Table 2 shows that the crude extracts were active on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigellia dysenteric*, *Candida albicans* and *Microsporum canis*, which means that the extracts were sensitive on the microbes while *Klebselia pneumonia*, *Vibrio cholerae* and *Trichophyton* sp shows resistance to the root extracts of the plant, which also means that the extract were not active on the microbes. These activities could be due to the presence of some very active bioactive component in the plant as compared to the control used against the organism as shown in Tables 2 and 3.

Table 2: Antimicrobial activities of the root extract

Test Organisms	Petroleum ether	Chloroform	Ethyl acetate	Methanol
S. aureus	S	S	S	S
E. coli	S	S	S	S
K. pneumonia	R	R	R	R
S. typhi	S	S	S	S
S. dysenteriae	S	S	S	S
V. cholerae	R	R	R	R
C. albicans	S	S	S	S
M. canis	S	S	S	S
T. sp	R	R	R	R

S = Sensitive; R = Resistance

Table 3: Control drugs against the test microorganisms

Test Organisms	Ciprofloxacin	Sparfloxacin	Fulcin	Fluconazole
Staphylococcus aureus	S	S	R	R
Escherichia coli	S	S	R	R
Klebselia pneumonia	R	S	R	R
Salmonella typhi	S	S	R	R
Shigellia dysenteriae	S	R	R	R
Vibrio cholerae	S	R	R	R
Candida albicans	R	R	R	S
Microsporum canis	R	R	S	R
Trichophyton sp	R	R	S	R

S = Sensitive R = Resistance

Table 4: Zone of inhibition (mm) of the extracts against the test microorganism

Test organisms	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Staphylococcus aureus	18	22	27	20
Escherichia coli	18	24	26	22
Klebselia pneumonia	0	0	0	0
Salmonella typhi	16	21	26	20
Shigellia dysenteriae	18	25	28	23
Vibrio cholerae	0	0	0	0
Candida albicans	18	21	24	20
Microsporum canis	16	20	24	18
Trichophyton sp	0	0	0	0

Table 5: Zone of inhibition (mm) of the drug against the test microorganism

Test Organisms	Ciprofloxacin	Sparfloxacin	Fulcin	Fluconazole
Staphylococcus aureus	30	32	0	0
Escherichia coli	37	34	0	0
Klebselia pneumonia	0	32	0	0
Salmonella typhi	41	35	0	0
Shigellia dysenteriae	38	0	0	0
Vibrio cholerae	29	0	0	0
Candida albicans	0	0	0	34
Microsporum canis	0	0	31	0
Trichophyton sp	0	0	30	0

Zone of inhibition (mm) of the root extracts against the test organism

The zones of inhibition of ethyl acetate extract gave the highest value, followed by chloroform extract, methanol extract and petroleum ether extract, respectively. This means that ethyl acetate root extract is very active on the organism while petroleum ether root extract is the least active on the organism as shown in the Table 4. It could be observed that when compare the value of zone of inhibition of each root extract to that of control drugs, their values were very close indicating the activeness of the extracts as shown in Table 5.

Minimum inhibition concentration (MIC) of the petroleum ether root extract

The MIC values were obtained thus; the petroleum ether root extract of *Cochlospermum planchonii* had value on *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigellia dysenteric, Candida albicans and Microsporum canis* at 2.5 mg/ml. while *Klebselia p Pneumonia, Vibrio cholerae and Trichophyton sp* show resistance to the extract as shown in Table 6.

Table 6: MIC of the petroleum ether root extract (mg/ml)

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	–	O*	+	++	+++
Escherichia coli	–	O*	+	++	+++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	O*	+	++	+++
Shigellia dysenteric	–	O*	+	++	+++
Vibrio cholerae	0	0	0	0	0
Candida albicans	–	O*	+	++	+++
Microsperum canis	–	O*	+	++	+++
Trichophyton sp	0	0	0	0	0

– = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Table 7: MIC of the chloroform root extract (mg/ml)

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	–	–	O*	+	++
Escherichia coli	–	–	O*	+	++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	–	O*	+	++
Shigellia dysenteric	–	–	O*	+	++
Vibrio Cholerae	0	0	0	0	0
Candida albicans	–	–	O*	+	++
Microsperum canis	–	–	O*	+	++
Trichophyton sp	0	0	0	0	0

– = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Minimum inhibition concentration (MIC) of the chloroform root extract

MIC values of the chloroform root extract was observed to inhibit the growth of *Staphylococcus aureus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigellia dysenteric, Candida albicans* and *Microsperum canis* at concentration 1.25 mg/ml and *Klebselia pneumonia, Vibrio Cholerae* and *Trichophyton sp* show resistance to the extract as presented in the Table 7. This was an indication that the fraction was very active.

Minimum inhibition concentration (MIC) of the ethyl acetate root extract

To determined further MIC of the root extract was carried out on ethyl acetate root extract. MIC values of the ethyl acetate root extract was observed to kill the growth of *Staphylococcus aureus* and *Shigellia dysenteric* at concentration 0.625 mg/ml. while *Escherichia coli, Salmonella typhi, Candida albicans* and *Microsperum canis* at concentration 1.25 mg/ml and *Klebselia pneumonia, Vibrio cholerae* and *Trichophyton sp* also show resistance to the ethyl acetate root extract. These results are showed in the Table 8.

Table 8: MIC of the ethyl acetate root extract (mg/ml)

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	–	–	–	O*	+
Escherichia coli	–	–	O*	+	++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	–	O*	+	++
Shigellia dysenteric	–	–	–	O*	+
Vibrio cholerae	0	0	0	0	0
Candida albicans	–	–	O*	+	++
Microsperum canis	–	–	O*	+	++
Trichophyton sp	0	0	0	0	0

Key: – = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Table 9: MIC of the methanol root extract (mg/ml)

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	–	–	O*	+	++
Escherichia coli	–	–	O*	+	++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	–	O*	+	++
Shigellia dysenteric	–	–	O*	+	++
Vibrio Cholerae	0	0	0	0	0
Candida albicans	–	–	O*	+	++
Microsperum canis	–	O*	+	++	+++
Trichophyton sp	0	0	0	0	0

Key: – = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Minimum inhibition concentration (MIC) of the methanol root extracts

The Minimum Inhibitory Concentrations (MIC) experiment of the crude root extract was equally determined further so as to know the activities of the extract. MIC values of methanol root extract was determined to exterminate the growth of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigellia dysenteric* and *Candida albicans* at concentration 1.25 mg/ml while *Microsperum canis* at concentration of 2.5 mg/ml and *Klebselia pneumonia, Vibrio cholerae* and *Trichophyton sp* was also showed resistance to the extract as shown in Table 9.

MBC/MFC of the petroleum ether root extract from

This was carried out so as to know if the organism is completely exterminated on just inhibited by the extracts. The MBC/MFC results was observed to be a little above the MIC values. The MBC/MFC value was observed to exterminate the *growth* of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigellia dysenteric, Candida albicans* and *Microsperum canis* at concentration of 5 mg/ml and *Klebselia pneumonia, Vibrio cholerae* and *Trichophyton sp* show resistance on the extract. This shows that there are bioactive substances in the petroleum ether extract that are active as show in the Table 10.

Table 10: MBC/MFC of the petroleum ether root extract (mg/ml)

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	O*	+	++	+++	+++
Escherichia coli	O*	+	++	+++	+++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	O*	+	++	+++	+++
Shigellia dysenteric	O*	+	++	+++	+++
Vibrio Cholerae	0	0	0	0	0
Candida albicans	O*	+	++	+++	+++
Microsperum canis	O*	+	++	+++	+++
Trichophyton sp	0	0	0	0	0

– = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

MBC/MFC of the chloroform root extract

The MBC/MFC for the chloroform root extract were observed for the following organism, *Staphylococcus aureus,*

Salmonella typhi, *Candida albicans* and *Microsperum canis* at concentration 5 mg/ml while *Escherichia coli* and *Shigellia dysenteric* at concentration 2.5 mg/ml which show that at this concentration chloroform extract can completely kill the organism. Generally the MBC/MFC for the chloroform extract had a value a little above that of the MIC value as observed in the Table 11.

Table 11: MBC/MFC of the chloroform root extract (mg/ml) of *Cochlospermum planchonii*

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	–	–	O*	+	++
Escherichia coli	–	–	O*	+	++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	–	O*	+	++
Shigellia dysenteric	–	–	O*	+	++
Vibrio Cholerae	0	0	0	0	0
Candida albicans	–	–	O*	+	++
Microsperum canis	–	–	O*	+	++
Trichophyton sp	0	0	0	0	0

– = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

MBC/MFC of the Ethyl Acetate Root Extract from *Cochlospermum planchonii*

These were determined to ascertain whether the organisms were only inhibited or are completely exterminated by the ethyl acetate root extract. It was observed that *Escherichia coli, Salmonella typhi, Candida albicans and Microsperum canis* were exterminated at 1.25 mg/ml while *Staphylococcus aureus and Shigellia dysenteric* were exterminated at 0.625 mg/ml as shown in the Table 12.

Table 12 MBC/MFC of the ethyl acetate root extract (mg/ml) of *Cochlospermum planchonii*

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococcus aureus	–	–	–	O*	+
Escherichia coli	–	–	O*	+	++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	–	O*	+	++
Shigellia dysenteric	–	–	–	O*	+
Vibrio cholerae	0	0	0	0	0
Candida albicans	–	–	O*	+	++
Microsperum canis	–	–	O*	+	++
Trichophyton sp	0	0	0	0	0

– = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

Table 13: MBC/MFC of the methanol root extract (mg/ml) of *Cochlospermum planchonii*

Test organisms	5	2.5	1.25	0.625	0.313
Staphylococus aureus	–	–	O*	+	++
Escherichia coli	–	–	O*	+	++
Klebselia pneumonia	0	0	0	0	0
Salmonella typhi	–	–	O*	+	++
Shigellia dysenteric	–	–	O*	+	++
Vibrio Cholerae	0	0	0	0	0
Candida albicans	–	–	O*	+	++
Microsperum canis	–	O*	+	++	+++
Trichophyton sp	0	0	0	0	0

– = No turbidity (no growth), O* = MIC, + = Turbid (light growth), ++ = Moderate turbidity, +++ = High turbidity

MBC/MFC of the Methanol Root Extract from *Cochlospermum planchonii*

This was determined so as to know if the extract can go further to kill the microorganisms which were sensitive to them. The MBC/MFC results was observed to be a little above the MIC value. It was observed that *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigellia dysenteric* and *Candida albicans* at concentration 1.25 mg/ml while *Microsperum canis* was observed at concentration 2.5 mg/ml and *Klebselia pneumonia*, *Vibrio cholerae* and *Trichophyton sp* show resistance to the extract as show in the Table 13.

Antioxidant qualitative assay

The spotted and sprayed TLC plates show resolved bands with color changes (yellow on purple background) due to the bleaching action of DPPH. By this it shows that the ethyl acetate and the methanol root extracts of the plant exhibit antioxidant activities.

Antioxidant quantitative assay

The extracts indicate the ability to function as antioxidants from the qualitative assay. Extracts of the root plant were found to reduce the DPPH radical. The antioxidant activities of the extracts were concentration dependent; that is, high concentration gives high percentage of DPPH inhibition with low inhibitory concentration (IC_{50}) value of 1.06 and 2.61 µg/ml of Methanol and ethyl acetate extracts, respectively.

The scavenging effects of the root extracts as compared with standard ascorbic acid are as shown in Table 14 and Fig. 1.

Table 14: Antioxidant screening of the ethyl acetate and the methanol root extracts

Concentration (µg ml^{-1})	%DPPH of ethylene acetate extract	%DPPH of methanol extract	%DPPH of ascorbic acid	Inhibition concentration (IC_{50}) for Methanol (µg ml^{-1})	Inhibition concentration (IC_{50}) for Ethyl acetate (µg ml^{-1})
100	71.42	73.79	107.08	1.06	2.61
200	65.94	80.99	107.08	1.06	2.61
400	77.99	88.96	107.08	1.06	2.61
500	64.42	82.38	107.08	1.06	2.61
800	52.78	81.80	107.08	1.06	2.61
1000	65.94	84.43	107.08	1.06	2.61

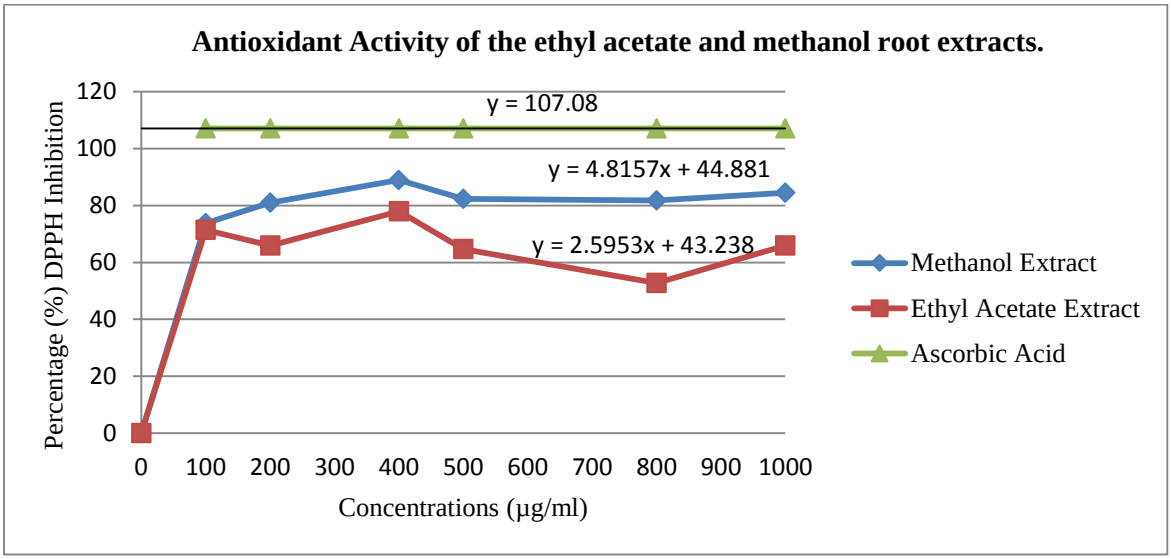

Fig. 1: Graph antioxidant of activity of root extracts of *Cochlospermum planchonii*

Conclusion

In conclusion, the root extracts of *Cochlospermum planchonii* has shown high biological activities and antioxidants properties. The stem leaf extracts had earlier been reported to contain tannins, flavonoids and other phenolic compounds (Isah *et al.*, 2013). The presence of these phytochemicals may account for the observed anti-oxidant activities of the plant.

Recommendation

This study has shown that the root extract of *Cochlospermum planchonii* possess high medicinal values as revealed by the antimicrobial screening and antioxidant analysis, thus it is recommended that the potential in these root plant should be established for possible industrial utilization as source of natural antimicrobial and antioxidants.

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Conflict of Interest

There are no conflicts of interest.

Reference

Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM & Farombi EO 2007. Phytochemical constituents and antioxidant activity of extract from the leaves of the *Ocimum graticcimum*. *Sci. Res. Essay*, 2: 163 - 166.

Barry AL & Thornsberry C 1985. Susceptibility tests, diffusion test procedure. *J. Chem. Pathol*., 19: 492-500.

Bauer AW, Kirby WMM, Sherris JC & Truck M 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., 45(4): 493-496.

Burton GW & Ingold KU 1989. Mechanisms of antioxidant action: preventive and chain-breaking antioxidants, In J. Miquel, A.T. Quintanilla, & H. Weber (Eds.). Handbook of free radicals and antioxidants in biomedicine (Vol. 2) Boca-Raton, FL: CRC Press, pp. 29.

Carini R, Poli G, Diazini MU, Maddix SP, Slater TF & Chessman KH 1990. *Biochemical Pharmacology*, 39: 1597-1601.

Ghani A 1990. *In Traditional Medicine*. Jahangirnagar University, Savar, Dhaka: p. 15 – 40.

Isah Y, Ndukwe IG & Ayo RG 2013. Phytochemical and antimicrobial analyses of stem-leaf of *Cochlospermum planchonii. Journal of medicinal and herbal Therapy Res.*, 1: 13-17. www.bluepenjournals.org/jmphtr.

Sadhu SK, Okuyama E, Fujimoto H & Ishibashi M 2003. Seperation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and anti-oxidant activities. *Chem Pharm Bull*., 51: 595-598.

Saha K, Lajis NH, Israf DA, Hamzah AS, Khorizah, S, Khamis S & Syahida A 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *J. Ethnopharmacol*., 92: 263 – 267.