

Phytochemicals, *in vitro* antioxidant, total phenolic contents and phytotoxic activity of *Cornus macrophylla* Wall bark collected from the North-West of Pakistan

Syed Muhammad Hassan Shah¹, Syed Muhammad Mukarram Shah*², Zakia Ahmad³, Muhammad Yaseen³, Raza Shah⁴, Abdul Sadiq², Shahzeb Khan² and Burhan Khan⁴

¹Department of Pharmacy, Sarhad University of Science and Information Technology

²Department of Pharmacy, University of Malakand, Malakand, KPK, Pakistan

³Department of Botany, University of Malakand, Malakand, KPK, Pakistan

⁴HEJ, Research Institute, University of Karachi, Karachi

Abstract: Plants are one of the precious creatures of Allah, producing a verity of useful bioactive compounds having definite pharmacological actions on human body. Keeping in view this idea, the methanolic extract from the bark of *Cornus macrophylla* was investigated for phytochemicals, antioxidant, total phenolic contents (TPC) and phytotoxic activities. Phytochemical analysis of *Cornus macrophylla* revealed the presence of tannins, anthraquinones, glycosides, reducing sugar, saponins and flavonoids. The percent free radicals scavenging potential of DPPH at 20, 40, 60, 80 and 100µg/ml was 72.69%, 73.32%, 73.51, 73.83% and 74.33% respectively and were compared to ascorbic acid (84.6%, 92.83%, 95.36%, 96.40% and 98.03%), gallic acid (85.49%, 92.47%, 95.14%, 98.22% and 98.03%) and quercetin (95.35%, 96.30%, 97.16%, 98.02% and 98.28%) as standards. The IC₅₀ value of *Cornus macrophylla* was 14.5/µg/ml. The TPC of the methanolic bark extract was 2.916 mg gallic acid equivalents/g of extract. The extract have shown excellent phytotoxic activity against the tested plant *Lemna minor* and inhibited the growth at 1000 µg/ml. Our findings revealed that the crude methanolic extract of *Cornus macrophylla* is a potential source of natural antioxidants and herbicidal.

Keywords: *Cornus macrophylla*, secondary metabolites, DPPH radical, TPC, Phytotoxic.

INTRODUCTION

The prehistoric humans did not know the basis of disease and started using medicinal plants for ailments based on their experience. After the birth of chemical sciences, use of medicinal plants was rationalized in the modern society. The practitioners of different eras tried to document this knowledge. This passion was continued and developments made in each time were conveyed to the successive generation. This healthy activity proved fruitful and aided to the drug armamentarium of humankind. The miracle of medicinal plants to alleviate pain and sufferings fascinated humans and they started systemic study of plants for human benefits. By this virtue a repertoire of medicinal agents have been isolated and reported from nature (Petrovska, 2010).

Free radicals when generated in the body causes various diseases such as cancer, diabetes by damaging proteins, lipids and DNA molecules. Various phytochemicals produced by plants have free radical scavenging properties like, flavonoids, carotenoids, alkaloids, rotenoids etc. In this way, these phytochemicals are responsible for countering deleterious effects of reactive oxygen species (ROS), the major factors responsible for formation of free radicals (Gutteridge and Halliwell, 1995).

One of the major obstacles in the high yield of crop production is the use of synthetic chemical herbicides. The most common flaws pertaining to the use of synthetic herbicides are the pest resistance and negative impact on natural enemies along with health and environmental related damages. These factors have increased the interest of the researchers to develop natural pesticides which could be effective, environment friendly and biodegradable as compared to the current synthetic pesticides (Li, 2010).

The *Cornus macrophylla*, Wall belongs to family *cornacea*. Its local name is khadang and is used for various infections. The *Cornus macrophylla* plant is a deciduous tree, rarely shrubs, growing to 15m (49ft) by 10m (32ft) tall (Noshiro and Baas, 2000).

Fruit of *Cornus macrophylla* is the ingredient of many traditional medicines and has been used as a remedy for the betterment of liver and kidney function. It is also used as analgesic, tonic, diuretic and for the preservation of foods. The fruit of *Cornus macrophylla* is being used for different ailments like malaria, allergy, infections, inflammation, diabetes, cancer and as lipid peroxidative (Kim and Kwak, 1998, Mau *et al.*, 2001, Gaw and Wang, 1949).

*Corresponding author: e-mail: shahpharmacist@yahoo.com

Keeping in view the medicinal importance of *Cornus macrophylla* the present work was designed to evaluate the phytochemicals, antioxidant, total phenolic contents and phytotoxic activities of this specie.

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh bark of *Cornus macrophylla* was collected from Hazarnau hill of Kot Manzary Baba, District, Malakand agency Khyber Pakhtunkhwa Pakistan during June 2011. The plant was identified by plant taxonomist, Assistant Professor Dr Nasrullah, Department of Botany, University of Malakand, Khyber Pakhtunkhwa Pakistan.

Extraction

After collection, the plant bark was washed with distilled water, cut into small fragments, dried under shade and grounded into a coarse powder. About 160g of powdered plant materials were soaked in 800ml methanol (80%) for a week. The extract was filtered using Whatman's filter paper. The filtrate was evaporated to dryness under reduced pressure at temperature of 40-45°C with rotary evaporator.

Phytochemical Screening

Chemical tests were carried out on methanol extracts of *Cornus macrophylla* bark using standard procedures to identify the phytochemicals as described by (Sofowora, 1982).

Alkaloids

About 0.2g of the extracts was warmed with 2% H₂SO₄ for two minutes on a boiling water bath. The mixture was then cooled, filtered and treated with the Dragendorff's reagent. Then observed the sample for the presence of orange red precipitation

Tannins

Small quantity of extract about 2g was mixed with 1ml water and heated on water bath. The mixture was filtered and 1-3 drops of ferric chloride solution was added to the filtrate and observed the sample for a dark green coloration, which shows the presence of tannins.

Anthraquinones

About 0.5g of the extracts was boiled with 2ml 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. After cooling 2ml of chloroform was added to the filtrate and 2-4 drops of 10% NH₃ were added to the mixture and heated, then observed the sample for the formation of rose-pink colour.

Glycosides

The extract was hydrolyzed by adding 5ml HCl solution and neutralized by adding 5/ml NaOH solution. After this a few drops of Fehling's solution A and B were added to the mixture and observed the sample for red precipitates.

Reducing sugars

About 5g of extracts was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for minutes. Then the sample was observed for orange red precipitate.

Saponin

About 0.2g of the extract was shaken with 5ml of distilled water and then heated to boil. Then the sample was observed for the frothing (appearance of creamy miss of small bubbles).

Flavonoids

Extract of about 0.2g was dissolved in 2ml diluted (2%) NaOH and made a solution (a) having yellow colour. After this 2% HCl was added to solution (a) and observed the sample for discoloration.

Phlobatannins

The extract 0.5g was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. After heating the sample were observed for red precipitate.

Terpenoids (Salkowski test)

About 0.2g of the extract of the plant sample was mixed with 2ml of CHCl₃ and 3ml concentrated H₂SO₄ was carefully added to form a layer. Then a reddish brown coloration in the interface will be observed.

DPPH Free radical-scavenging activity

The hydrogen atom or electron donation abilities of the corresponding extracts and standards were measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Experiments were carried out according to the method of Blois (Blois, 1958) with a slight modification. Briefly, a 1mM solution of DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample solutions in ethanol (containing 20-100µg) and control (without sample). After 30min, the absorbance was measured at 517nm. Decrease in the absorbance DPPH solution indicates DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follow:

$$\% \text{ RSA} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

The tests were carried out in triplicate and the results were expressed as mean values \pm standard deviations. The extract concentration showing 50% inhibition (EC₅₀) was calculated from the graph of % RSA against extract concentration with Quercetin, Ascorbic acid and Gallic acid used as standards.

Determination of Total Phenolic compounds

An antioxidant compound generally contains Phenolic group (s). Briefly, 1ml of each of the already prepared

extract solution (500µg) was transferred into a 50ml volumetric flask and volume was adjusted to 45ml by addition of distilled water. Afterwards, 1ml of Folin-Ciocalteu Reagent (FCR) was added into this mixture and after 3min, 3ml of Na₂CO₃ (2%) was added. Subsequently, mixture was shaken for 2hr at room temperature and then absorbance was measured at 760nm. Phenolic compounds were quantified in triplicate (Biglari, 2008). The results were mean values ±standard deviations and expressed as mg of Gallic acid equivalents µg/mg of extract (GAEs) by using an equation that was obtained from the standard curve, is given as:

$$A = 1156C + 0189$$

Where A is the absorbance and C is the Gallic acid equivalent (mg/g). In this assay, 500µg of dried extracts were added to test samples, and final volumes were 50ml.

Phytotoxic activity

In vitro phytotoxicity assay was carried out for the crude extract and subsequent solvent dilution against *Lemna minor*. The medium was prepared by mixing various inorganic components in 100ml of distilled water and KOH solution was added for the adjustment of pH at 6.0-7.0. The medium was sterilized in autoclave at 121°C at 15psi pressure for 15min. Test samples (10mg) were dissolved in solvent (ethanol 1ml), used as stock solution. Three flasks for each dilution were inoculated with 10, 100 and 1000µl of the stock solution for 5, 50 and 500ppm of the extract. Under sterile conditions the solvent was evaporated overnight. Each flask was supplemented with 20ml of the medium. Thereafter, 10 plants each containing a rosette of three fronds, were added to each flask. One other flask, supplemented with solvent as control and reference plant growth inhibitor (Paraquate), served as a standard phytotoxic drug. The flasks were plugged with cotton and placed in growth cabinet for seven days. On the seventh day, the numbers of fronds per flask were counted (McLaughlin, 1991). Results were analyzed as growth regulation in percentage, calculated with reference to the negative control.

RESULTS

Phytochemical screening

During this study various phytochemicals were detected in the plant materials. The results are shown in the Table 1. Their presence has been indicated with positive (+) mark and absence with negative (-) mark in the table. Phytochemical screening of *Cornus macrophylla* bark, have shown a verity of phytochemicals i.e., tannins, anthraquinones, glycosides, reducing sugar, saponin, flavonoids and terpenoids. However alkaloids and phlobatanins were not detected by their respective reagents.

DPPH Free radical-scavenging activity and Total phenolic contents

As can be seen from Table 2, the potent antioxidant activity is concentration dependent. Different concentrations of *Cornus macrophylla* bark methanolic extract show DPPH free radical scavenging activity as 72.69%, 73.32%, 73.51%, 73.83% and 74.33% at 20, 40, 60, 80 and 100µg/ml respectively, with an IC₅₀ value of 14.5µg/ml. In the present study the antioxidant activity of *Cornus macrophylla* were also compared with quercetin, gallic acid and ascorbic acid as shown in table 2. The % RSA of quercetin is remarkably higher than *Cornus macrophylla* and the values are more close to the gallic acid and ascorbic acid. It has been noted that the % RSA value of *Cornus macrophylla* is less than that of the compared standards on the lower concentration but significant differences were observed at higher concentration.

Phytotoxicity of *Cornus macrophylla*

In our study *Cornus macrophylla* have shown highly significant activity against the tested plant *Lemna minor* at a dose of 1000µg/ml. When different concentration (10, 100 and 1000µg/ml) of the extract were applied the result obtained showed moderate to excellent phytotoxic activity i.e. 5, 65 and 95% respectively as shown in the table 5.

Table 1: Phytochemical screening of crude methanolic extracts of *Cornus macrophylla* bark.

| S. No. | Chemical component | Extract | Reagent/Chemical | PR/AB | Observation |
|--------|--------------------|---------|---|-------|---|
| 1 | Alkaloids | 0.2 g | Dragen droff's | - | Orange red preceptation not found |
| 2 | Saponins | 0.2 g | D. Water | + | Frothing |
| 3 | Flavonoids | 0.2 g | NaOH+HCl | + | Decolouration |
| 4 | Terpenoids | 0.2g | CHCl ₃ +H ₂ SO ₄ | + | Redish brown colouration of the interface |
| 5 | Anthraquinones | 0.5 g | HCl+CHCl ₃ +NH ₃ | + | Rose pink colour |
| 6 | Phlobatanins | 0.5 g | HCl | - | Red preceptate not found |
| 7 | Tannins | 2 g | Ferric chloride | + | Dark green colouration |
| 8 | Glycosides | 5 g | Fehling's solution | + | Red preceptate |
| 9 | Reducing sugar | 5 g | Fehling's solution | + | Orange red preceptation |

Table 2: Comparison of % RSA of *Cornus macrophylla* (C.M) with Quercetin (Q), Ascorbic acid (A.A) and Gallic Acid (G.A)

| Test Solution Sample/ standard | Concentration µg/ml | | | | |
|--------------------------------------|---------------------|-------|-------|-------|-------|
| | 20 | 40 | 60 | 80 | 100 |
| C.M | 72.69 | 73.32 | 73.51 | 73.83 | 74.33 |
| Q | 95.35 | 96.3 | 95.36 | 98.02 | 98.28 |
| A.A | 86.22 | 92.83 | 95.36 | 96.4 | 97.6 |
| G.A | 85.49 | 92.47 | 95.14 | 98.03 | 98.22 |

Table 3: Total Phenolic Contents (TPC) of crude methanolic extract of *Cornus macrophylla* bark

| Control | Sample A | Sample B | Sample C | Mean | C |
|---------|----------|----------|----------|-------|-------|
| 0.038 | 0.344 | 0.365 | 0.359 | 0.356 | 2.916 |

DISCUSSION

Phytochemicals are naturally occurring compounds of plant kingdom, which work with nutrients and fibers to act against diseases or more specifically provide protection against diseases (Devasagayam *et al.*, 2004).

These are secondary metabolites produced by the plants and are used as drugs, food additives, pesticides, flavours and fragrances. These natural substances are in clinical use today. In the modern world, scientists are struggling to enhance their production inside the plants by various techniques due to their importance (Hussain, 2012). It is worth mentioning that mostly in experiments whole plant extracts are used to show their therapeutic effects but studies are available in which individual classes of secondary metabolites are subjected to pharmacological assays. Antimicrobial, analgesic, anti-inflammatory, anticancer activities have been reported for flavonoids (Comalada *et al.*, 2006, Mamadalieva *et al.*, 2011), glycosides (Dembitsky, 2005), tannins (Serrano *et al.*, 2009, Mossi *et al.*, 2009) etc. Saponins have shown anticancer activity (Auyeung *et al.*, 2009), antimicrobial activity (Avato *et al.*, 2006), analgesic and anti-inflammatory activity (Moharram and El-Shenawy, 2007). We found flavonoids, glycosides, tannins, saponins, anthraquinones etc in our study. This shows the range of therapeutic potential of this plant, *Cornus macrophylla*.

Medicinal plants are rich source of free radical scavengers, because they contain antioxidants, which can slow, terminate or retard the rate of oxidation reaction by

Table 4: Phytotoxic activity of methanolic extract of the bark of *Cornus macrophylla*

| Name of Plant | Conc. of extract µg/ml | No. of Fround | | %Growth Regulation | Conc. of Std. Drug µg/ml |
|---------------|------------------------|---------------|---------|--------------------|--------------------------|
| | | Sample | Control | | |
| Lemna minor | 1000 | 1 | 20 | 95 | 0.015 |
| | 100 | 7 | 20 | 65 | |
| | 10 | 9 | 20 | 5 | |

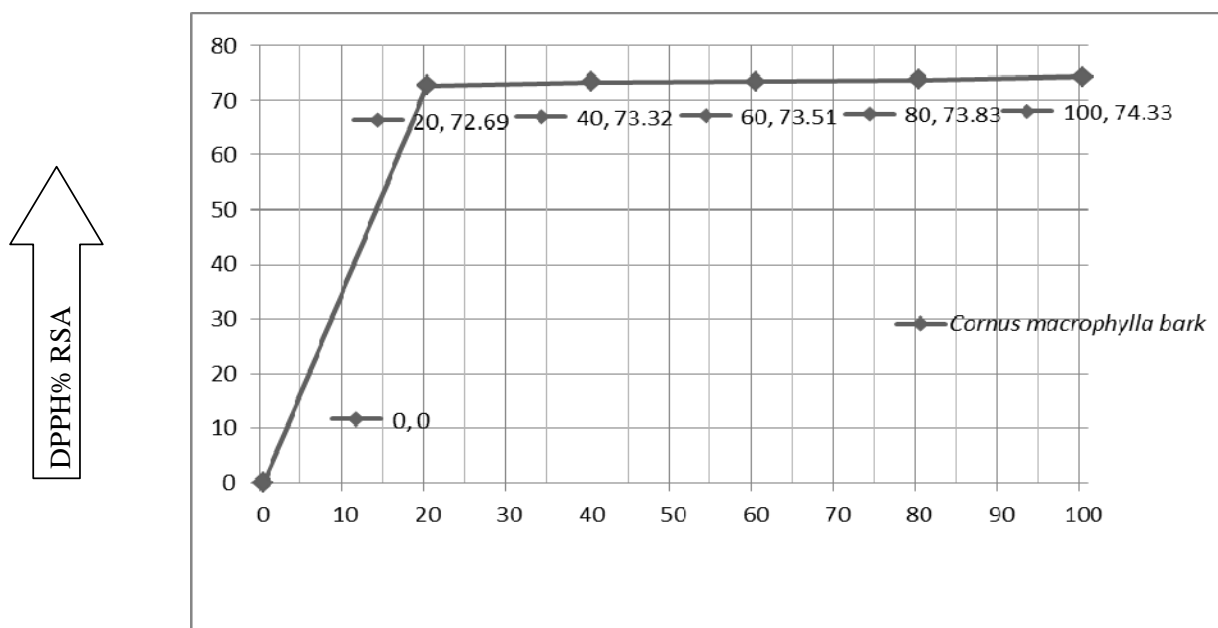


Fig. 1: % RSA graph of *Cornus macrophylla* bark for IC50 value.

scavenging free radicals. These antioxidants have been implicated as causative agents of more than 100 diseases and also ageing. Nature has gifted natural machinery for preventing generation of ROS by providing cells with enzymes like superoxide dismutase and catalase. After generation, these ROS are made ineffective by scavenging process by antioxidants (Devasagayam *et al.*, 2004). Others inhibit the oxidation reaction by removing oxygen free radicals.

Phenolic compounds are the largest group of phytochemicals and responsible for antioxidant activity of plants or plant products (Ji *et al.*, 2011). Flavonoids were found in the methanolic extract of *Cornus macrophylla*. Flavonoids protect against oxidative stress. These chemicals scavenge peroxy radicals effectively due to their good reduction potentials relative to alkyl peroxy radicals and thus are effective inhibitors of lipid peroxidation. Presence of B-ring catechol group (dihydroxylated B-ring) is responsible for hydrogen donating ability of flavonoids and hence scavenging of reactive radical species (Rice-Evans, 2001, Polovka *et al.*, 2003). Antioxidant activity of this plant may be due to presence of flavonoids, tannins and other phenolic contents.

Human population is increasing at very high rate and is forecasted to be 10 billion by the year 2050. Different strategies should be developed in order to provide normal quantity of food to each individual. The main obstacle in the crops production is the diseases of crops, caused by pests and weeds. Most of the medicinal plants produce numerous phytochemicals, which insures their survival by protecting them against infections (Luthria *et al.*, 1993, Ciccina *et al.*, 2000). In our study, the results obtained are in agreement with other phytotoxic studies on plants (Nisar *et al.*, 2009).

REFERENCES

- Auyeung KK, Cho CH and Ko JK (2009). A novel anticancer effect of Astragalus saponins: Transcriptional activation of NSAID-activated gene. *Int. J. Cancer*, **125**: 1082-1091.
- Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z and Jurzysta M (2006). Antimicrobial activity of saponins from medicago sp: Structure-activity relationship. *Phytother Res.*, **20**: 454-457.
- Blois MS (1958). Antioxidant determinations by the Use of a Stable Free Radical. *Nature*, **181**: 1199-1200.
- Ciccina G, Coussio J and Mongelli E (2000). Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J. Ethnopharmacol.*, **72**: 185-189.
- Comalada M, Ballester I, Bailon E, Sierra S, Xaus J, Galvez J, DE Medina FS and Zarzuelo A (2006). Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: Analysis of the structure-activity relationship. *Biochem. Pharmacol.*, **72**: 1010-1021.
- Dembitsky VM (2005). Astonishing diversity of natural surfactants: 5. Biologically active glycosides of aromatic metabolites. *Lipids*, **40**: 869-900.
- Devasagayam T, Tilak J, Bolor K, Sane KS, Ghaskadbi SS & Lele R (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. *J. Assoc Physicians India*, **52**: 794-804.
- Gaw HZ and Wang HP (1949). Survey of Chinese Drugs for Presence of Antibacterial Substances. *Science*, **110**: 11-12.
- Gutteridge JMC and Halliwell B (1995). Antioxidants in Nutrition, Health and Disease, Oxford University Press, USA.
- Hussain MS, Fareed S, Ansari S, Rahman MA, Ahmad IZ and Saeed M (2012). Current approaches toward production of secondary plant metabolites. *J. Pharm Bioallied Sci.*, **4**: 10-20.
- Ji L, Wu J, Gao W, Wei J, Yang J and Guo C (2011). Antioxidant capacity of different fractions of vegetables and correlation with the contents of ascorbic acid, phenolics and flavonoids. *J. Food Sci.*, **76**: 1257-1261.
- Kim DK and Kwak JH (1998). A furan derivative from *Cornus officinalis*. *Arch Pharm Res* **21**: 787-789.
- Li ZH, Wang Q, Ruan X, Pan CD and Jiang DA Phenolics and plant allelopathy. *Molecules*, **15**: 8933-8952.
- Luthria DL, Ramakrishnan V and Banerji A (1993). Insect Antifeedant Activity of Furochromones: Structure-Activity Relationships. *J. Nat. Prod.*, **56**: 671-675.
- Mamadaliyeva NZ, Herrmann F, EL-readi MZ, Tahrani A, Hamoud R, Egamberdieva DR, Azimova SS and Wink M (2011). Flavonoids in *Scutellaria immaculata* and *S. ramosissima* (Lamiaceae) and their biological activity. *J. Pharm. Pharmacol.*, **63**: 1346-57.
- Mau J, Chen C and Hsieh P (2001). Antimicrobial effect of extracts from Chinese chive, cinnamon and corni fructus. *J. Agric Food Chem.*, **49**: 183-188.
- Moharram FA and EL-shenawy SM (2007). Antinociceptive and anti-inflammatory steroidal saponins from *Dracaena ombet*. *Planta Med.*, **73**: 1101-1106.
- Mossi AJ, Mazutti M, Paroul N, Corazza ML, Dariva C, Cansian RL Oliveira JV (2009). Chemical variation of tannins and triterpenes in Brazilian populations of *Maytenus ilicifolia* Mart. *Ex. Reiss. Braz. J. Biol.*, **69**: 339-345.
- Nisar M, Tariq SA, Marwat IK, Shah MR and Khan IA (2009). Antibacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on *Indigofera gerardiana*. *J. Enzyme Inhib. Med. Chem.*, **24**: 224-229.

- Noshiro S and Baas P (2000). Latitudinal trends in wood anatomy within species and genera: Case study in *Cornus s.l.* (Cornaceae). *Am. J. Bot.*, **87**: 1495-1506.
- Petrovska BB (2010). Historical review of medicinal plants' usage. *Pharmacogn. Rev.*, **6**: 1-5.
- Polovka M, Brezova V and Stasko A (2003). Antioxidant properties of tea investigated by EPR spectroscopy. *Biophys. Chem.*, **106**: 39-56.
- Rice-evans C (2001). Flavonoid antioxidants. *Curr. Med. Chem.*, **8**: 797-807.
- Serrano J, Puupponen-pimia R, Dauer A, Aura AM and Saura-calixto F (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nutr. Food Res.*, **53**(2): 310-129.
- Sofowora A (1982). *Medicinal Plants and Traditional Medicine in Africa*. John Wiley & Sons, New York, 256pp.