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PHYTOCHEMICAL AND PHARMACOGNOSTICAL ANALYSIS OF *FLUEGGEA LEUCOPYRUS* WILLD

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ABSTRACT

Herbal medicines as the major remedy in traditional system of medicine. The aim of the present study was to investigate the various phytochemicals and pharmacognosy of *Flueggea leucopyrus* Willd aerial parts. The aerial parts powder were extracted with petroleum ether, chloroform and methanol successively. Phytochemical analysis shows the presence of alkaloids, steroids, saponin, catachin, tannins and cardiac glycoside. Methanolic extracts showed more secondary metabolites. The generated data from the three different extracts of *Flueggea leucopyrus* aerial parts provided the basis for its wide uses in the traditional & folk medicines. The pharmacognosy study includes organoleptic characters and microscopic characters along with estimation of its physicochemical parameters such as ash values, water soluble ash, acid insoluble ash, sulphated ash, extractive value, moisture content and preliminary phytochemical screening. The present study would provide the helpful information for the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

KEYWORDS: *Flueggea leucopyrus*, Phytochemistry, Pharmacognosy.

INTRODUCTION

India has rich diversity of medicinal plants and from ancient times these plants were utilized as therapeutic agents. Western Ghats is very rich in its medicinal wealth. The forests and hills of this region is a treasure house of about 700 medicinal plants. Today's research is mainly

focused on medicinal plants because the bioactive compounds and medicinal power mainly depends on phytochemical constituents that have great pharmacological significance. The phytochemical constituents, natural bioactive compounds, nutrients and fibers present in medicinal plants, fruits and vegetables defend us from various ailments.[1] Phytochemicals are classified into two major groups namely primary constituents like amino acids, sugars, proteins and chlorophyll etc., secondary constituents includes alkaloids, essential oils, flavanoids, tannins, terpenoids, saponins and phenolic compounds etc., more over they bear valuable therapeutic activities.[2]

Flueggea leucopyrus Willd. is medicinally important plant belonging to Euphorbiaceae family and is a thorny woody shrub. The plant is sweet, cooling, diuretic, aphrodisiac, tonic useful in vitiated conditions of Pitta, burning sensation, strangury, seminal weakness and general debility. Leaves act as a disinfectant (antiseptic) and its paste is used by the tribes to extract any extraneous materials from body tissues without surgery. Paste of *Flueggea leucopyrus* leaves mixed with tobacco is used to destroy worms in sores.[3] The leaves were boiled and taken orally twice a day for stomach ache.[4] The plant has been used in preparations in traditional medicine for the treatment of cough, hay asthma, bowel complaints, disinfections, laxatives, diarrhoea, gonorrhoea, constipation , mental illness and kidney stones.[5, 6] The leaves are also used in the treatment of piles and fibroids.[7] The roots are used in the treatment of testicular enlargement and in the cure of edema. The whole plant is used for the cure of cancer in the sole of the foot.[7] It is also used in the treatment of abdominal lumps and liver hypertrophy and portal hypertension. The bark of stem is used for tooth ache.[7] The main objective of the present study is to analyze the various phytochemical constituents and pharmacognosy of *Flueggea leucopyrus* Willd aerial parts.

MATERIALS AND METHODS

Collection of plant Materials

Healthy aerial parts of *Flueggea leucopyrus* Willd were collected from foot hills of Western Ghats, Sivanthipuram. The specimen was authenticated by Dr. M. Johnson, Assistant Professor, Department of Botany, St. Xavier's College and the voucher specimen (XCH 26879) was deposited in St. Xavier's College Herbarium, Palayamkottai.

Extract Preparation

About 60g of air-dried, coarsely powdered aerial parts of *Flueggea leucopyrus* Willd were extracted using petroleum ether, chloroform and methanol through Soxhlet method by

successive extraction. The extraction was carried out about 72 hours. The extracts were concentrated using vacuum evaporator and analyzed for further analysis.

Preliminary Phytochemical screening

Phytochemical screening of petroleum ether, chloroform and methanol extracts were done by standard procedure.[8, 9]

Pharmacognostic studies

Macroscopic studies

The macroscopic characters such as size, shape, margin, apex, surface, colour, odour, taste, nature, texture were studied for morphological investigation.[10]

Microscopic studies

The leaves, stem and root of *Flueggea leucopyrus* Willd were cut and fixed in FAA (formalin 5 mL+ acetic acid 5 mL + 70% ethyl alcohol 90 mL). The specimens were cast into paraffin blocks. The paraffin embedded specimens were section with the help of rotary microtome. The thickness of the sections was 10-12 µm. The section was then stained with safranin and Fast- green.

Physico-Chemical Character

Quantitative Determination

The percentages loss of weight on drying, total ash, water soluble ash, acid insoluble ash and sulphated ash residue on ignition were obtained by employing standard methods of analysis as described in pharmacopoeia of India (1966).[11]

Determination of Total Ash

5 g of dried powder of aerial parts of *Flueggea leucopyrus* Willd were taken in a previously weighed silica crucible and ignited carefully not exceeding dull red heat until the ash was free from carbon. The crucible was cooled and weighed. The percentage of ash with reference to the air-dried plant was calculated.

Determination of water-soluble ash

A known weight of ash was boiled with 25 mL of distilled water. The insoluble matter was collected in previously weighed sintered crucible. The crucible was washed with water, dried and weighed. The percentage of water soluble ash with reference to the air-dried plant was calculated.

Determination of acid -insoluble ash

A known weight of ash was boiled with 25 mL of dilute hydrochloric acid. The insoluble matter was collected in previously weighed sintered crucible. The crucible was washed with hot water, dried to attain constant weight. The percentage of water soluble ash with reference to the air-dried plant was calculated.

Determination of sulphated ash

5 g of the air-dried and powdered aerial parts of *Flueggea leucopyrus* Willd were taken in a silica crucible and moistened with concentrated sulphuric acid. It was ignited gently and moistened with concentrated sulphuric acid and then re-ignited. The crucible was cooled and weighed. The percentage of sulphated ash was calculated with reference to the air-dried sample.

Extractive value

The different extracts of aerial parts of *Flueggea leucopyrus* Willd were weighed and the percentage of extractive value were calculated with reference to the air-dried powdered sample.

Moisture content

5 g of fresh plant sample was weighed in a pre-weighed silica crucible. It was dried in the oven at 105℃ and weighed at intervals of one hour until two successive constant weights were obtained. The loss of weight was recorded as moisture content.

Fluorescence Analysis

For analytical studies, air-dried powdered aerial parts of *Flueggea leucopyrus* Willd and the various extracts using solvents such as petroleum ether, chloroform and methanol were examined under Visible light and Ultra Violet light (UV-365 nm). The powder was treated with various chemical reagents such as 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid, 50% nitric acid, acetic acid, acetone, ethanol, water and ammonia. The fluorescence behavior were recorded and tabulated.

RESULTS AND DISCUSSION

Phytochemistry

Phytochemical and proximate analyses of plants are used to assess their potential nutritive and medicinal benefits. The phytochemical screening demonstrated the presence of different

types of secondary metabolites like alkaloids, steroids, phenol, saponin, tannin, catachin and cardiac glycoside.

Table 1: Preliminary Phytochemical screening of different extracts of aerial parts of *Flueggea leucopyrus* Willd

Metabolites	Pet. Ether	Chloroform	Methanol
Alkaloid	-	-	+
Steroid	+	+	+
Phenol	+	+	+
Flavonoid	-	-	-
Saponin	+	+	-
Tannin	-	-	+
Anthraquinone	-	-	-
Terpenoid	-	-	-
Catechin	-	-	+
Carbohydrate	-	-	-
Cardiac glycoside	+	+	+
Coumarin glycoside	-	-	-

Petroleum ether extracts showed four metabolites such as steroid, saponin, phenol and cardiac glycoside. Chloroform extracts confirmed the presence of steroid, saponin, phenol and cardiac glycoside. Methanol extracts showed more bioactive compounds such as alkaloids, steroids, phenol, tannin, catachin and cardiac glycoside.

Secondary metabolites are chemicals produced by plants. Secondary metabolites can be classified on the basis of chemical structure, composition, their solubility in various solvents. A simple classification includes three main groups: terpenes, phenolics and nitrogen containing compounds. Alkaloids have antitumor, anti-amoebic, anti plasmodial, anti fungal, anti-bacterial, anti-ulcer and anti-feedant activities. Alkaloids comprised the largest single class of secondary plant substances. They have a remarkable range of pharmacological activity. The pharmacological studies in alkaloids have been largely concerned with effect of alkaloids on physiological processs other than inflammation. Steroids are known to be an important Cardio tonic activities posse's antimicrobial property and also used in herbal medicines and cosmetics. Tannins have antibacterial, antidote, haemostatic, mild diuretic, stomachic and insecticidal properties. Saponnins are known to possess anti mutagenic, anti fungal, hypocholesterolic, anti cancerous, anti cytotoxic, zenotoxic and clastogenic, anti inflammatory, heamolytic, immunoadjuvent, anti protozoal, spermicidal activities. Tannins protect against microbiological degradation of dietary proteins in the semen. Glycosides possess anti-HIV, antiaxidater, anti-leukaemia, antidiabetic, cardiac, molluscidal,

antibacterial, analgesic, antipyretic, aphrodisiac, laxative and antistress characters. Saponins have been reported to control human cardiovascular diseases and can also decrease cholesterol. These are surface active agents which interfere with or alter the permeability of the cell wall whereas phenols have bactericidal, anti-oxidant, anti-microbial, anti-viral, anti-inflammatory and vasodilatory effects.[12] The presence of cardiac glycosides are known to play a major role in heart muscles by inhibiting Na+ and K+ pump that increase the availability of sodium ions and calcium ions to heart muscles which improves cardiac output and reduce heart distension. Thus are used in the treatment of congestive heart failure and cardiac arrhythmia.[13]

Pharmacognosy

Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained.

Macroscopic Characters

Shrubs, branchlets angular, ending in spines. Leaves distichous, to 2.5 x 1.5 cm, obovate, apex emarginate, base cuneate, membranous. Flowers greenish white; bracts many, minute, pedicels 2 mm; male flowers in axillary clusters, tepals 0.7 mm, concave, obtuse; stamens 5, filaments 1.5 mm, pistillode 1 mm. Capsule 5 mm across, globose, white.

Fig. 1. Morphology of *Flueggea Leucopyrus* Willd

Microscopic properties

Leaf

The leaf is dorsiventral with prominent midrib and bilaterally symmetrical lamina. The midrib is slightly raised on the abaxial side and semicircular on the abaxial side. The epidermal layers of the midrib consist of small thick walled cells. A single prominent vascular strand is placed in the centre of midrib which is supported adaxially and abaxially by

compact, thick walled, hyaline parenchymatous ground tissue. The vascular bundle is radial, with short row of thick walled wide and circular xylem elements and a narrow arc of phloem strands. The lateral veins also have prominent vascular strand. They do not project much beyond the level of the lamina.

Lamina

The lamina is circular with adaxial epidermal cells; some of the cells having dense mucilage contents. The abaxial epidermis has large papillate cells which are 20 µm thick. The mesophyll tissue is differentiated into adaxial zone of dense, darkly stained, two or three layers of palisade cells, a median row of dilated, angular hyaline parenchyma cells and an abaxial zone of small, lobed mostly vertically oriented spongy parenchyma. There is another layer of dilated hyaline parenchyma cells just above the abaxial epidermis. The leaf margin is thick and slightly curved abaxially. Fig.2 showed the epidermis of Leaf.

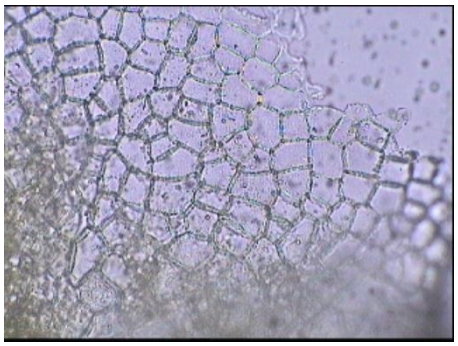

Fig.2. Leaf epidermis.

Petiole

The petiole has a narrow, distinct layer of epidermis with circular, thick walled cells. The ground tissue consists of dilated, compact fairly thick walled parenchyma cells. The cells contain large calcium oxalate druses; each cell has single druses filling the entire cell lumen. The druses are also seen in abundance in the mesophyll tissue. The vascular strand is single, prominent and semicircular. It has a several, parallel rows of xylem elements and an arc of phloem.

Young Stem

The young stem is circular in outline with a thin layer of intact epidermis. Two layers of sub epidermal cells have elongated radially forming a wide zone of oblong cells. The phellogen is seen along the inner boundary of the elongated cortical cells. The cortex is narrow

comprising of 3-4 layers of parenchyma cells. The inner boundary of the cortex has a thick, discontinuous cylinder of sclerenchyma cells. Secondary phloem is wide and continuous. Secondary xylem consists of long radial vessels, narrow xylem rays and xylem fibres. The vessels are wide with 15-25 µm in diameter. They are thick walled and angular in diameter. The xylem fibres are thick walled and lignified with wide lumen. The rays are straight and spindle shaped (Fig.3).

Old stem

In the old stem, the epidermis is broken at a several places due to vigorous expansion of periderm. In the broken region, the phloem is exposed. The phloem cells are in 4-5 layers; they are radially oblong and thick walled. A distinct phellogen and two layers of phelloderm are seen in the periderm. Secondary xylem is increased in the radial width. It consists of heavily thick walled fibres arranged in regular radial rows; the lumen is fairly wide. The xylem rays are one or two cells wide, straight and the ray cells are thick and lignified. The fibres at certain places are gelatinous type. The vessels are in short and long radial multiples; they are angular or ovate, thick walled and wide.

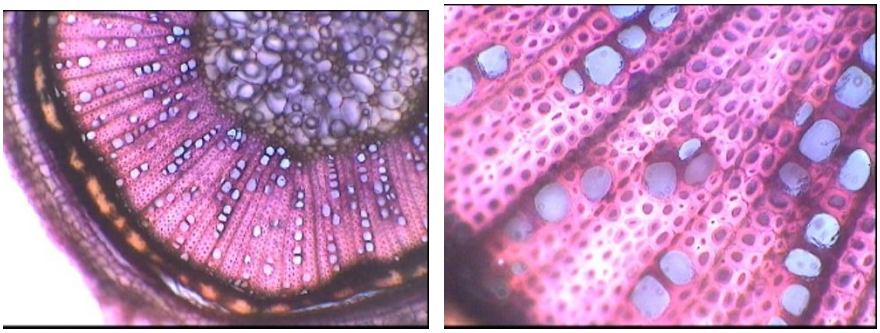

Fig. 3. T.S of Stem

Root

Internal structure of *Flueggea leucopyrus* root consists of the following features. Epiblema is the outermost layer of the root. It is also known as rhizodermis or piliferous layer. It is uniseriate comprising tubular living components. Unicellular root hairs are formed due to elongation of cells in epiblema. Root hairs are present in maturation zone of root. Cuticle and stomata are absent. The second layer of root is called **c**ortex which is made up of parenchymatous cells. The cortex is followed by **e**ndodermis. This layer is situated between the pericycle and cortex. Casparian strips are present on radial and tangential wall of endodermis. These strips are made up of suberin. The cells of endodermis which are situated

in front of protoxylem cells lack of casparian strips. These are called passage cells / transfusion cells. The number of passage cells is equivalent to the protoxylem cells. Passage cells provide path to absorbed water from cortex to pericycle. Vascular bundles are radial and exarch. Xylem and phloem are separate and equal in number. The number of xylem bundles is two to six (diarch to hexarch). Parenchyma cells which is found between xylem and phloem is called conjunctive tissue. Pith is less developed or absent (Fig. 4).

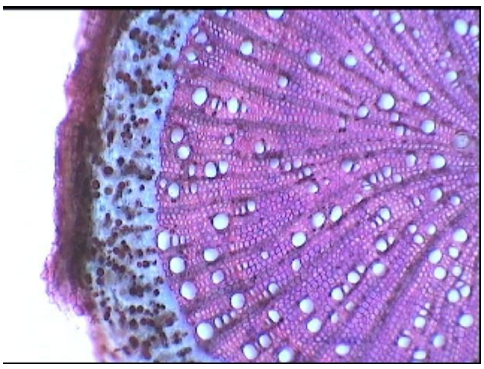

Fig. 4. T.S. of Root

Physicochemical parameters

The results of physico parameters like total ash, water soluble ash, acid insoluble ash, sulphated ash, extractive value and moisture content of *F. leucopyrus* were tabulated in table 2.

Table 2: Physicochemical parameters of *Flueggea leucopyrus* Willd aerial parts

Parameters	Weight percentage (%)
	Aerial parts
Total Ash	3.08
Water soluble ash	0.84
Acid insoluble ash	2.65
Sulphated ash	5.53
Extractive values	
Petroleum ether	1.18
Chloroform	0.75
Methanol	13.07
Moisture Content	
Aerial parts	31.62

Adulteration is a rampant phenomenon since adulterants also share a number of characters with the genuine drugs and therefore it becomes imperative to recognize them in the raw material, powder or extract form. Total ash values and extractive values were useful in identification and authentication of the plant material. Extractive values were useful to

evaluate the chemical constituents of crude drugs. The total ash values were higher than the water soluble ash, acid insoluble ash and lower than sulphated ash values. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing.[14] Thus all the physicochemical parameter of plant drugs reported here for the first time, is an important for detecting adulteration or improper handling of drugs which would be useful in standardization of herbal drugs.

Fluorescence Analysis

The fluorescence analysis of the different extracts and powder of *Flueggea leucopyru*s aerial parts was given in Table 3. The powder was treated with various reagents and the mixture was observed under UV light (365 nm) to see the fluorescence behavior. Fluorescence analysis is one of the pharmacognostical procedures useful in the identification of authentic samples and recognizing adulterants.[15] The present study is very useful aid to confirm authenticity and to check adulterants in crude form.

Table 3: Fluorescence Analysis of *Flueggea leucopyrus* Willd

	Aerial Parts	
	Visible	**UV**
Pet. ether extract	Brown	Dark Green
Chloroform extract	Dark Brown	Dark Green
Methanol extract	Dark Brown	Dark Green
Powder	Light Brown	Pale Green
Powder + 1N NaOH	Dark Brown	Dark Green
Powder + 1N HCl	Pale Brown	Pale Green
Powder + 50% H_2SO_4	Reddish Brown	Dark Green
Powder + 50% HNO_3	Reddish Brown	Dark Green
Powder + CH_3COOH	Yellow	Pale Green
Powder + Acetone	Light Brown	Pale Green
Powder + Ethanol	Pale Brown	Pale Green
Powder + Water	Pale Brown	Pale Green
Powder + Ammonia	Brown	Dark Green

Fluorescence analysis is one of the pharmacognostical procedures useful in the identification of authentic samples and recognizing adulterants. In the present study, the fluorescence analysis was evaluated for *F. leucopyrus* aerial parts in their powdered form or in different solvents and reagents. Although, in most of the cases the actual substances responsible for the fluorescence properties has been identified, the merits of simplicity and rapidity of the process makes it a valuable analytical tool in the identification of plant samples and crude drugs.[16]

Determination of minerals

A number of inorganic elements are present in ash of chosen plant material. The existence of such inorganic elements in plants can be detected qualitatively. The qualitative determinations of inorganic minerals in ash are tabulated in table 4.

Table 4: Qualitative analysis of ash minerals of *F. leucopyrus* Willd

S.No	Minerals	Aerial parts
1	Chlorides	-
2	Sulphate	+
3	Phosphate	+
4	Iron	-
5	Calcium	-
6	Potassium	+
7	Magnesium	-
8	Cobalt	-
9	Copper	-

In plants, the inorganic elements are available only in trace amounts which may usefully influence various functions. These elements are used extensively in chemotherapy and are essential in human and animal health.[17 - 19] Some of the chemical compounds and elements found in the extracts have been known to exert pharmacological effects, while others are capable of protecting the active ingredients in the herb from decomposing either chemically or physiologically.[20]

In plants the majority of sulphur is assimilated in the reduced form.[21] Sulphur is an essential component of vitamins, biotin and co-enzyme.[22 –24] Phosphorous, as phosphate is an integral component of plant cells which maintains blood sugar level, normal heart contraction[25], bone growth and kidney function when consumed by humans.[26] Calcium, magnesium and potassium are essential for making good of worn out cells, building of red blood cells and maintaining body mechanisms.[27] Sodium and potassium take part in ionic balance of human body and help in the formation of gastric juice in stomach.[28]

CONCLUSION

The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. The preliminary phytochemical screening reveals the presence of alkaloids, steroids, tannins, phenols, catachin, saponin and cardiac glycoside in various extracts of *F. leucopyrus* aerial parts. Pharmacognosy and physicochemical characters to analyze their quality, safety and standardization for their safe

use. The observed information of the present study will provide data which is helpful in the correct Identification, authentication of this medicinal plant and may help in preventing its adulteration.

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REFERENCE

1. Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO. Phytochemical constituents and antioxidant activity of extract from the leaves of the Ocimum graticcimum. Sci. Res. Essays, 2007; 2: 163-166.
2. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J. Med. Plants Res., 2009; 3(2): 67-72.
3. Solangaarachchi SM. and Perera BMS. Floristic composition and medicinally important plants in the under storey of the tropical dry mixed evergreen forest of the Hurubu reserve of Srilanka. J. National Sci. Counc. Srilanka, 1993; 2: 209-221.
4. Suresh KR, Kottaimuthu R, Norman SJ, Kumuthakalavalli R, Simon SM. Medicinal plants used by Malayali tribals in Kolli hills of Tamilnadu. Int. J. Res. Ayurveda Pharm., 2011; 2: 502-508.
5. Donda MR, Rao K, Alwala J, Miryala A, Sreedhar B, Pratap MP. Synthesis of silver nanoparticles using extracts of *Securinega leucopyrus* and evaluation of its antibacterial activity. Int. J. Curr. Sci., 2013; 7: 1-8.
6. Jayaweera DM. Medicinal plants (Indigenous and Exotic) Used in Ceylon-part-2. The National Science Foundation of Sri Lanka, 1980.
7. Anonymous. A Sri Lankan study of compendium of medicinal plants. Aurvedic department Sri Lanka; 2001; pp 152-155.
8. Brindha P, Sasikala B, Purushothaman KK. Pharmacognostic studies on Merugan Kizhangu. Bull. Med. Eth. Bot. Res., 1981; 3: 84-96.
9. Harborne JB. "Phytochemical Methods," Chapman and Hall Ltd., London, 1973; 49-188.
10. Jahan N, Afaque NA, Ahamad G, Ansari AA. Physicochemical studies of the Gum Acacia. Indian Journal of Natural Product Resources, 2008; 7: 335-7.
11. Anonymous, "Indian Pharmacopoeia" Govt. Of India, Ministry of Health, Controller of Publication, Delhi, India., 1996.

12. Aletor VA. Allelochemicals in plants and feedingstuffs. Nutritional, biochemical and physiological aspects in animal production. Vet. Hum. Toxicol., 1993; 35: 57-67.
13. Schneider G and Wolfling J. Synthetic Cardenolides and related compounds. Current Organic Chemistry, 1993; 8: 14.
14. Mukherjee PK. Quality Control of Herbal Drugs. New Delhi, India: Business Horizons. 2002.
15. Saini ML, Saini R, Roy S, Kumar A. Comparative pharmacognostical and antimicrobial studies of Acaciaspecies (Mimosaceae). Journal of Medicinal Plants Research, 2008; 2(12): 378-386.
16. Tyler, V.E., Brady, L.R. and Robbers, J.E. "Pharmacognosy", Lea & Febiger, Philadelphia, 1976; 24.
17. Khan IZ. The role of chemistry in health, disease and aging. A Seminar paper presented in the Department of Chemistry, University of Maiduguri, Maiduguri. 1996.
18. Ogugbuaja VO, Akinniyi JA, Abdulrahman FI and Ogarawu VC. Elemental contents of medicinal plants. A monograph, Department of chemistry, faculty of science, University of Maiduguri, Nigeria. 1997.
19. Moses EA, Ogugbuaja VO, Ogarawu VC. Enrichment of element of Nigerian bituminous coal fly ash and their effects on hematological parameters of exposed rabbits. Nig. J. Exp. Applied Biol., 2002; 3: 95 – 100.
20. Abdulrahman FI and Onyeyili PA. Phytochemical screening and pharmacological activities of the stem bark of Terminaliaavicennoides. Bull. Anim. Health Prods. Afr., 2001; 49: 236-242.
21. Bick JA and Leustek T. Plant sulphur metabolism- the reduction of sulphate to sulphite. Current Opinion in Plant Biology, 1998; 1: 240 – 244.
22. Herry JB and Statland BE. Chemical pathalogy and clinical chemistry, W.B. Saunders Company, Philadelphia. 1979.
23. Chouhan F, Wattoo MHS, Tirmizi SA, Menon FZ, Rahman A and Tufail M. Analytical investigation of inorganic nutritive elements of Capparis decidua grown in Cholistan desert. The Nucleus, 2002; 39(3-4): 195-199.
24. Tirmizi SA, Wattoo FH, Wattoo MHS, Kanwal S and Iqbal J. Inorganic Nutrients of Saccharumbengalense. Chem. Soc. Pak., 2005; 27: 186 – 189.
25. Linder and Manria, C. Nutritional biochemistry and metabolism with clinical applications. Appleton and Lange, Norwalk, 1991; 2: 191 - 212.

26. Johns T and Duquette M. Deficiency of phosphorus in man. Am. J. of clin. Nutr., 1991; 53: 448 -456.
27. WHO. Trace Elements in Human Nutrition and Health. World Health Organization, Geneva, 1996.
28. Brody T. Nutritional Biochemistry, San Diego Academic press, 1998; 11 –12.