Pharmacognostical Evaluation of Leaf and Stem Extract of Parkia Biglandulosa Wight & Arn

L.P. Khalid[1], P.V. Pulate[2], Kanchan Mate[3]
[1,2,3]Department of Botany, Vidya Bharati Mahavidyalaya Amravati (MH), India

Abstract—**This work aimed to quantify plant parts of Parkia biglandulosa like leaf and stem. In the current work investigation carried out in terms of Ethno-medical uses Organoleptic evaluation, anatomy, powder microscopy, extractive value, Chemical behavioural analysis, phytochemical investigation, quantification and TLC of both the plant parts. P. biglandulosa is used against bronchitis, pneumonia, diarrhea, violent colic, vomiting, sores and ulcers. Organoleptic evaluation carried out by color, odor, taste and special features including texture for drug identification. Rachis of P.biglandulosa showed presence of unicellular trichomes and T.S of stem showed hairy fiber cuticles. In powder microscopy cork cell, fiber, trichomes, starch grains observed. Qulitative and quantitative phytochemical analysis of stem and leaf showed presence of various secondary metabolites in different solvent. TLC Chromatography showed various bands and RF value.**

Index Terms—**Organoleptic & Extractive value evaluation, Phyarmacognosy, TLC, Anatomy and P.biglondulosa.**

I. INTRODUCTION

The genus Parkia (Fabaceae, Subfamily, Mimosoideae) comprises about 34 species of mostly evergreen trees widely distributed across neotropics, Asia, and Africa [1] active of Malaya. The genus is named after Mungo Park. It is a large handsome, evergreen tree. Flowers are small, ball-shaped, and brown initially turning to white, in pendant flower heads on long stalks. Propagation is by seeds [2]. Pharmacognosy, in recent years has gained immense importance as it is an efficient tool for the authentication and identification of plant raw materials and therefore evaluation of pharmacogenetic parameters is an indispensable step when dealing with herbal drugs [3].

Phytochemical metabolites are certainly found in the therapeutic plant parts like root, leaves, bark, vegetables and fruits that have express mechanism and protect from innumerable diseases [4]. The different parts of the plant show antiulcer activity, anti-inflammatory activity [5,6] antibacterial activity, antifungal activity, Malarial fever, skin diseases [7] etc. The stem bark is reported to contain tannin, saponins, glycosides, sterols and triterpenoids [8,9]. Saponin's from the seed bran of Parkia biglandulosa is used medicinally in India for its astringent and detergent properties and also as a fish poison [10].

II. MATERIAL METHOD

Parkia biglandulosa was collected during the winter season from botanical garden of Vidya Bharati Mahavidylaya Amravati region. Following parameters were done with Parkia biglandulosa plant part.

A. Organoleptic evaluation: Organoleptic evaluation of the drug refers to the evaluation of drug by colour, odour, taste and special features including texture [11].
B. Anatomical study: The collected fresh material of selectedplants was washed 2-3 times with sterile distilled water and preserved 3% formalin solution then used for the anatomical investigation and prepared double stain permanent slide for anatomical study and it observed under Carl Zeiss standard universal Microscope [11].
C. Powder microscopy: The plant drug contains some basic cell type. A little quantity of powder was taken onto a microscopic cavity slide.1-2 drops of 0.1% phologlucinol solution and a drop of conc.HCL were added, mount it in glycerol, covered with a cover slip and observed under the microscope with 10x10 magnification and characteristic features of plant powder were recorded. Powder microscopy was carried out by using the method mentioned in ayurvedic Pharmacopeia of India 1966.

D. Extractive Value: The procedures recommended in ayurvedic Pharmacopeia of India (1966) were followed for calculating extractive values.

E. Chemical behavioral analysis: This analysis was carried out by using the standard method mentioned in Ayurvedic Pharmacopeia of India (1966). Behavior of powdered plant materials with different chemical reagents i.e. conc. H 2 SO4, HNO3, HCL, 10% NaOH, Iodine solution, Ferric chloride, Potassium iodide,1N H_2SO_4, HNO_3, HCL were observed under day light and UV light.

F. Phytochemical test:

The methods used for detection of various phytochemical were followed by qualitative and quantitative chemical test to give general idea regarding the nature of constituents present in crude material of Parkia biglandulosa [12,13,14,15]

A} Qualitative analysis

1. Tests for carbohydrates

i) Fehling's Test: 1ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, the brick red precipitate was observed.

ii) Benedict's reagent: Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

iii) Keton's test: 2 ml extract add with few crystals of resorcinol and equal voloume of conc. Hcl and heat over sprit lamp observed rose coloration indicate presence of keton

2. Test for proteins

i) Million's Test: To the small quantity of extract 1-2 drops of Million"s reagent was added Formation of white colour precipitate showed presence of proteins.

ii) Test for Xanthoprotein: In 2ml of test solution a few drops of conc. nitric acid and 3ml of ammonia were added appearance of red precipitate indicate the presence of xanthoprotien.

3. Tests for Anthroquinone glycosides

Borntrager's Test: To the 3 ml of extracts, dil. H2SO4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

4. Tests for Cardiac glycosides

Keller-Killiani test: 5ml of extract, 1ml of conc.H2SO4, 2 ml of Glacial acetic acid and 1 drop of FeCl3 solution was added. Appearance of brown ring shows the presence of cardiac glycosides.

5. Test for steroids

Salkowski test: To 2 ml of extract, 2ml of chloroform and 2ml of conc.H2SO4 was added. The solution was shaken well. As a result, chloroform layer turned red and acid layer showed greenish yellow fluorescence.

6. Test for alkaloid

i) Mayer's test: 2-3 ml of filtrate, dil. HCl and Mayer"s reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

ii) Dragendroff's test: 2-3 ml of filtrate, few drops of dil. HCl and Dragendroff"s reagent was added and shake well. Formation of orangebrown precipitate showed the presence of alkaloids.

iii) Wagners test: 2 ml extract add the reagent reddishbrown ppt show indicates the presence of alkaloids.

7. Tests for flavonoids

iv) Lead Acetate test: To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

v) Alkaline test: To the test solution add few drops of NaOH solution formation of intence yellow color which turn to colorless solution after addition of dil. Acetic acid indicate presence of flavonoids.

vi) NaOH test: Small amount of extract was treated with NaOh and Hclobserve formation of yellow orange color.

vii) H2SO4: Fraction of extract treated with Conc.H2SO4 and observe formation of orange color.

8. Test for Saponin: Foam Test 1 ml extract 20 ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1 cm layer of foam was formed.

i) Frothing test: 3 ml extract with 10 ml D/W in test tube and plug the Stoppard and shake vigorously for 5 min. it allows to stand 30 min observe the honey comb forth indicate result.

ii) Test for Glycosides: The extract was mixed with a little anthrons on a watch glass. One drop of conc. sulphuric acid was added and made into a paste and warmed gentle over the water bath. Dark green coloration indicates the presence of glycosides.

iii) Test for Quinone: 2 ml test solution was treated with a few drops of conc. H_2SO_4 or aq. NaOH solution. Colour formation indicates the presence of quinoid compound.

9. Test For Lignin

i) Labat test: Equal volume of extraction solution mix with the gallic acid in a test tube. Its shows a olive green colour.

B} Quantitative analysis:

Quantitative analysis carried out by using standard data given by [16] for Total Phenolic Content (TPC) and [17] for Total Flavonoid Content (TFC).

G. Chromatographic analysis: The chromatographically study was carried out by using the standard procedure described [13,14] The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of Rf value.

Distance traveled by the solute from the origin

Resolution factor (Rf) = -----------------------------------

Distance traveled by the solvent from the origin

III. RESULT AND DISCUSSION

Classification:

Division: Spermatophyta

Class: Dicotyledoneae

Order: Fabales

Family: Fabaceae

Sub-Family: Mimosoideae

Genus: Parkia

Species: biglandulosa

Parkia biglandulosa, A large tree. Bar grey. Leaves 2-pinnate; rachis grooved, pinnae 20-40 pairs; leaflets 40-90 pairs/ linear-oblong, small, 0.2-0.3 cm, closely set. Flowers white; in long peduncled globose heads. Pods 15-20cm long, flat, reddish-brown, dehiscent, many seeded. Frequently planted in gardens and along roadsides in towns [18]

(Fig. 1 & 2)

Fig. 1Habit of Parkia biglandulosa

Fig. 2 fruit of Parkia biglandulosa

T.S of rachis showed parenchymatous unicellular trichomes on adaxial surface, followed by epidermis continue with 4-5 layered cortex. Elongated, collateral and open vascular bundle. Large vessels are observed in xylem tissue. Fig.3. T S. of stem showed circular outline. hairy trichomes at outer side followed by thick cuticle. Next to the cuticle, epidermis. 3-4 layered 3schlrenchymatous cortex followed by single layered pericycle and endodermis next to it. Centrally located vascular bundle which having xylem and phloem Fig. 4 similar structure also observed by [19].

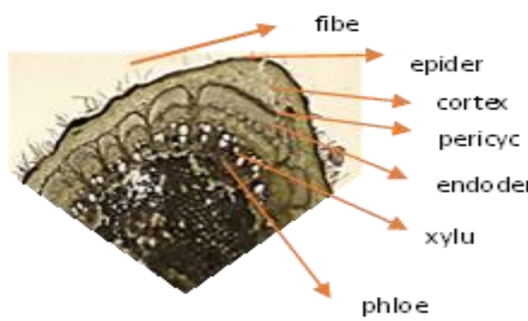

Fig. 3 T.S rachis of P. biglondulosa

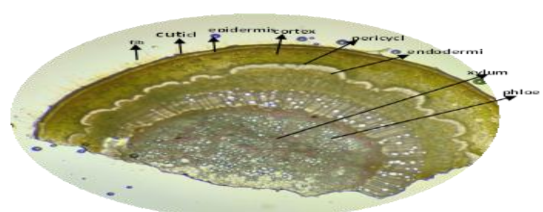

Fig. 4 T.S stem of P. biglondulosa

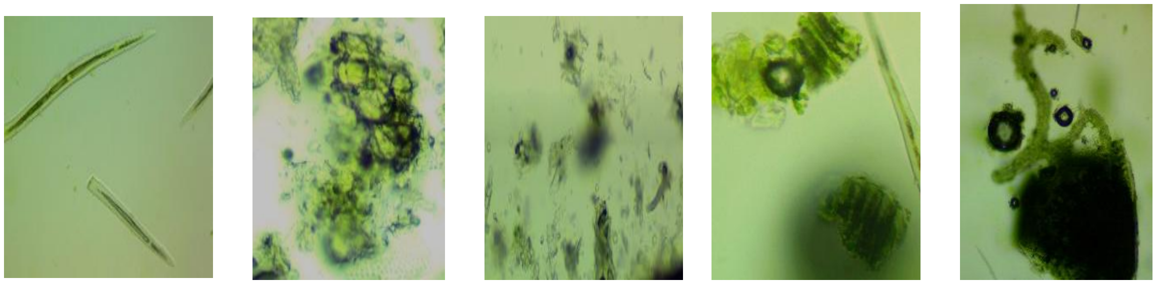

Fig 5 A- trichome B-epidermal cell C-starch grain D-cork cell E-fiber

Organoleptic evaluation of stem and leaves show similar odor and taste characters but color and texture are somewhat different Table No. 1 [20], observed that the bitter taste indicates that the powdered Pawpaw peel contain alkaloids while the sweet taste of the powdered pulp indicates the presence of glucose. In powder microscopy the aerial parts showed cork cell, trichomes, fiber in leaf and epidermal cell, starch grain in stem Table No.2 also saw by [21]. Chemical behavioral analysis showed various fluorescence effect in UV light and visible light Table no. 3. The result of phytochemical analysis present in table no.4. stem phytochemistry showed protein, alkaloid, saponin and tannin in chloroform, petrolium ether and benzene while leaf showing carbohydrate, alkaloid, protein, tannin, saponin and quinone in different solvent test. In leaf and stem protein were observed in petrolium ether. In stem alkaloid observed in chloroform, PE and benzene whereas in leaf it presents only in PE and benzene, according to [22] Alkaloids were only detected in the ethanol leaf extract.

The quantitative analysis of P.biglandulosa leaf and stem revealed the present of Total phenolic content (TPC) and total flavonoid Content (TFC) on different wavelength like 765nm for TPC and 415nm for TFC. leaf showing high TPC absorbance 2.092nm and stem had 1.548 nm, [19] observed terpenoid and phenolic value was 1.737 ± 0.149 % contents, amongst all the phytochemical fractions extracted, leaves of P. biglandulosa were found to be rich in quaternary alkaloids and n-oxides fraction. On the contrary, the fraction of fats and waxes i.e. the neutral extract was least. In TFC stem showed high absorbance0.621nm s compare to leaf (0.434nm) fig.no. 6. TLC profiling of methanolic extract for alkaloid and phenol observed different RF values with number of bands. Highest bands and RF values were observed for alkaloid in leaf methanolic extract i.e. 5bands with 4.5 RF value followed by phenol in leaf and alkaloid in stem having similar result that is 3bands and 0.37 RF values (fig no. 6 & 7), [19] Chromatographic evaluation- For HPTLC, mobile phase composition of toluene: methanol (8:1, v/v) showed a good resolution of β-sitosterol from other phytoconstituents (Figure 8). The Rf value of β-sitosterol was found to be 0.46. In case of HPLC, β-sitosterol eluted at the Rt of 6.8 min using the mobile phase of acetonitrile: ethanol (40:60, v/v).

Fig no.6 Quantitative analysis of stem and leaf of *P. biglandulosa*

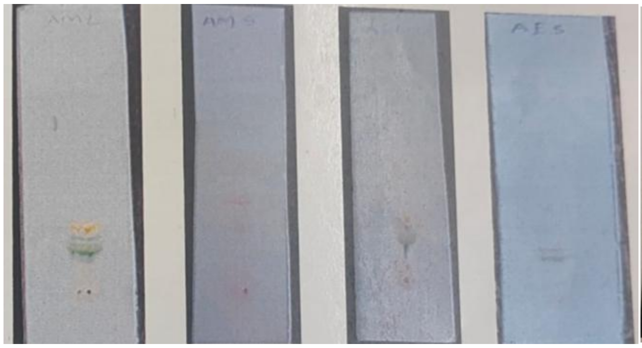

Fig no.7 Chromatographic analysis of methanolic leaf extract of *P. biglandulosa*

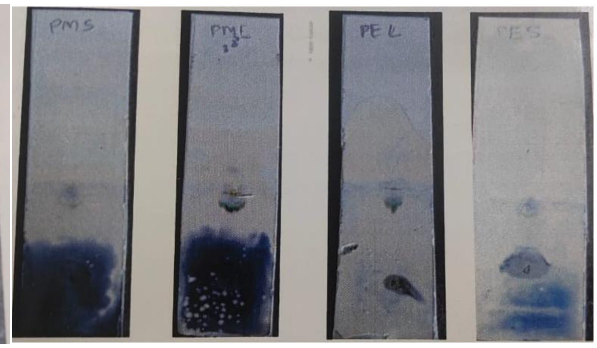

Fig no.8 Chromatographic analysis of methanolic stem extract of *P. biglandulosa*

Table No. 1 Organoleptic Evaluation of dried powder of P. biglandulosa

Sr no.	Particular	Plants parts	
		Stem	Leaves
1	Colour of powder	Light green	Cream
2	Odour	Mild	Mild
3	Tests	Bitter	Bitter
4	Texture	smooth	rough

Table no. 2 Powder microscopy

Sr.no	Plant part	Observed character
1	Leaf	Cork cell, trichomes, fiber
2	stem	Epidermal cell, starch grain

Table no. 3 Chemical behavioral analysis:

Test for fluorescence	stem		Leaf	
	Daylight color	U.V light color	Daylight color	U.V light color
Powder+1N HCL	Reddish brown	Dark brown	Dark green	Dark green
Powder + 1N NaOH	Black	Black	Dark green	Dark green
Powder+50% HNO3	Orange	Dark orange	orange	orange
Powder+ al.1 N NaOH	Black	Black	Dark green	Blackish green
Powder+50% H2SO4	Black	Black	Black	Black
Powder + methanol	Light greenish cream	Light greenish cream	Olive green	Dark green
Powder + water	Cream	Cream	Olive green	Green
Powder as such	cream	cream	Olive green	Light green

Table no.4 Qualitative Phytochemical screening of Leaf & Stem of P. biglandulosa Wight & Arn.

Sr no	Phytochemicals	Test name	LEAF				STEM			
			C	P	B	M	C	P	B	M
1.	Carbohydrate	Fehling	+	-	-	-	+	-	-	-
		Benedict	-	-	+	+	+	+	+	-
2.	Best for combined	Keton test	-	+	-	+	-	+	-	-
3.	Protein test	Xantho protein test	+	+	+	+	-	+	-	-
		Millon's	-	-	-	+	+	+	-	-
4.	Alkaloid	Mayers	+	+	+	-	-	+	+	+
		Dragandroff	-	-	-	+	-	+	+	-
		Wagners	+	+	+	-	+	+	+	-

5.	Phenolic	Carotenoids	-	-	+	-	-	+	+	-
		Potassium dichromate	-	+	+	+	-	-	+	+
		Fecl3	-	-	-	+	+	-	+	+
6.	Tannin	Gelatine	-	-	-	+	+	-	-	+
		Pholobtannin	-	-	-	+	-	-	-	-
		Braymer's test	-	-	-	+	-	-	-	+
		10% NaOH	+	+	+	-	+	+	+	-
7.	Cardiac glycoside	Keller- killani test	-	+	-	+	-	-	+	+
8.	Steroid	Salkowshi test	+	+	-	+	-	+	-	+
		H2SO4	-	+	-	-	-	-	-	+
		Fecl3	-	-	-	+	-	-	-	+
9.	Test for flavonoids	H2sO4	-	+	-	+	-	-	-	-
		Lead acetate	-	+	+	+	-	+	-	+
		NaOH	-	-	+	+	-	-	+	-
		H2SO4	-	+	-	+	-	-	-	-
10.	Saponin	Foam	-	-	-	+	-	-	-	+
		Frothing	+	+	+	-	-	+	+	-
11.	Quinone	Quinone	+	-	-	+	-	+	+	+
		Alcohlic KOH	-	-	-	+	+	-	-	-
		Sulphuric Acid	-	-	-	+	-	-	+	-
12.	Anthraquinone	Borntrager's test	-	-	-	+	-	-	-	+
13.	Lignin	Labat	-	-	-	-	-	-	-	-

(Where, C= Chloroform, PE= Petrolium ether, B= Benzene, M=Methanol, + Present, - Absent)

Table no.5 TLC profile of methanolic extract of P.biglandulosa

Sr.no.	Plant parts	Chemical constituents	Running solvent	RF value	Spraying reagent	Total bands
1	Stem	Alkaloid	Methanol+ chloroform	0.37	Dragandroffs reagents	03
		phenol	Chloroform+ acetic acid	0.37	FCR reagent	02
2	leaf	Alkaloid	Methanol+ chloroform	4.5	Dragandroffs reagents	05
		phenol	Chloroform+ acetic acid	0.37	FCR reagent	03

ACKNOWLEDGEMENT

Authors are sincerely thankful to the Head department of botany Dr. P.G. Bansod for providing laboratory facilities and all needful help.

REFERENCES

[1] Mohammed S M Saleh [1], Juriyati Jalil [2], Satirah Zainalabidin [3], Ahmad Yusof Asmadi [4], Nor Hidayah Mustafa [2], Yusof Kamisah (2021) . Genus Parkia: Phytochemical, Medicinal Uses, and Pharmacological Properties [1,] Int J Mol Sci. Jan 9;22(2):618. doi: 10.3390/ijms22020618.

[2] Rupesh Pingale1*, Deepak Pokharkar1, Suvarna P Phadatare2, Archana Gorle2 (2016). Pharmacognostic Evaluation of Parkia biglandulosa bark International Journal of Pharmacognosy and Phytochemical Research; 8(7); 1160-1163 ISSN: 0975-4873.

[3] Khare V, Kushwaha P, Verma S, Gupta A, Srivastava S. (2012) Pharmacognostic evaluation and

[4] antioxidant activity of Urtica dioica L. Chinese Medicine; 3: 128-35.

[5] Yogashree G.D. 1, Shivakumar Singh P. 2 and Shrishail H.C. (2021) Phytochemical Screening and Gc-Ms Analysis of Root Extracts of Parkiabiglandulosa (WIGHT&ARN.) Plant Archives Volume 21, No 1, pp. 314-323, e-ISSN:2581-6063 (online), ISSN:0972-5210.

[6] Rupesh Pingale, Deepak Pokharkar, S.C. Pal, G KDash. (2013) Anti-inflammatory and antiulcer activity of methanolic extract of bark

of Parkia biglandulosa on albino rats (wistar). IJPRD; 4(11).
[7] Samnt,A.R., J.P.Tiwari,D.V.Derle,S.B.Kasture,V.S.Kasture and S.C.Pal,(1998.Antiulcer activity of Parkia bigladulosa, Indian drugs, 35: 204-207.
[8] L T Ajibade, P O Fatoba, U A Raheem and B AOdunuga. (2004) Department of Geography, Universuty of Ilorin, Ilorin, Nigeria,
[9] Krasuss G.Y.J., and Reinbothe H., (1973)"Phytochemistry",, 12(1), 125-142.
[10] Gmelin R., Susilo R., and Fenwick G.R.,(1981) "Phytochemistry", , 20(11), 2521-3.
[11] Rupesh P, Pal SC, Pavani A, Gadge MS. (2010) Quantitativeestimation of the active constituents of Parkia biglandulosa by using HPTLC and FTIR. International Journal of Pharma and Bio Sciences; 1 (4): 315-22.
[12] Pharmacopoeias-- India: Indian Pharmacopoeias. India. Indian Pharmocopoeia Committee, Published by Delhi: Manager of Publication 1966 2 nd Edition.
[13] Kokate, C.K. Purohit,A.P.And Gokhale, S.B. (2005). Pharmacognosy, Nirali Publication Pune.
[14] Harborne, J.B. (1998) Phytochemical Method. 3rd Edition., Chapman and Hall Publication, London.
[15] Sadashivam And Manickam, A. (2005). Biomedical Methods 2nd Edn.New Age International (P) Ltd., Publisher, New Delhi.
[16] Wallis, T.E. (1990). Text Book Of Pharmacognosy. 5th Edn, CBS Publishers And Distributors.
[17] Singleton, V.L., lamuela- Raventos (1999); Analysis of total henols and other oxidation susbtrates and antioxidants by means of Folin-Ciocalteu reagent methods Enzymol. 1999, 299, 152-178. [CrossRef].
[18] Suman Kumar Ratnampally and Venkateshwar Chinna (2017); Quantitative analysis of phytochemicals in the Bark etracts of Medicinally important plant Cassia fistula Linn.
[19] M.A. Dhore 1986. Flora of Amravati district with special reference to the distribution of tree species.
[20] Sunita Shailajan, 1Neelam Sayed, 1Bhavesh Tiwari, 2Naresh Chandra (2013) Pharmacognostical Evaluation of Leaves of Parkia biglandulosa Wight & Arn.: An Important Ethnomedicinal Plant. International Journal of Pharmacognosy and Phytochemical Research; 5(2); 84-91 ISSN: 0975-4873. Available online on www.ijppr.com.
[21] Lahai Koroma, Dolly Anna Gandhi and Victoria Lebbie (2023). An Investigation Into Organoleptic Evaluation and Phytochemical Screening of Dried Powdered Peel, Pulp of Ripped Pawpaw, Production Fresh Pawpaw Juice and Sensory Evaluation of the Pawpaw Juice. International Journal of Advanced Engineering and Management Research Vol. 8, No. 01; 2023 ISSN: 2456-3676.
[22] Karuna Modi, Mamta Shah: A Complete Pharmacognostical Profile of Rungia repens, Pharmacogn J. (2017); 9(2): 123-127.A multifaceted peer reviewed journal in the field of Pharmacognosy www.phcogj.com | www.phcog.net
[23] Bukar, A., 2 Uba, A. and 1 Oyeyi, T.I (2010)) Phytochemical Analysis and Antimicrobial Activity of Parkia Biglobosa (Jacq.) Benth.Extracts Againt Some Food – Borne Microrganisms. Advances in Environmental Biology, 4(1): 74-79, 2010 ISSN 1995-0756 © 2010, American-Eurasian Network for Scientific Information.