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PHYTOCHEMICAL INVESTIGATION OF THE ETHANOL, METHANOL AND ETHYL ACETATE LEAF EXTRACTS OF SIX CASSIA SPECIES

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ABSTRACT

Establishment of pharmacognostic profile of the leaves will assist in standardization of quality, purity and sample identification. A comparative study of the secondary metabolites was performed on *Cassia alata*, *Cassia surratensis*, *Cassia occidentalis, Cassia auriculata, Cassia sericea and Cassia tora*. Evaluation of the leaf extracts using various solvents like ethanol, methanol and ethyl acetate was carried out to detect the presence of active components. Qualitative analysis was done for various constituents like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, saponins, tannins, cardiacglycosides and phlobatannins. The phytochemical screening revealed the presence of all the above chemicals except saponins, anthraquinones, anthocyanosides and phenolic flavonoids in some extracts.

KEY WORDS

Cassia species, leaf extracts, medicinal importance, secondary metabolites, solvents.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine.(**Anjali.D.Ruikar, 2009**). The topography of India which is in the tropical belt with its varied climatic zones makes it a vast storehouse of medicinal plants. There is a continuous and urgent need to discover new compounds with diverse chemical structures and novel mechanisms of action (**A.Panneerselvam, 2011**). Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (**Ahmed, L.1998**). Over 50% of all modern clinical drugs are of natural product origin and natural clinical products play an important role in drug development programmes in the pharmaceutical industries.

Phyto-chemical investigations of crude plant extracts shows the presence of active principles in the plant parts like bark, leaves, flowers, roots, fruits, seeds etc. Phyto-chemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but research works demonstrates that many phyto-chemicals can protect humans against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

In the present work, qualitative phyto-chemical analysis was carried out in six species of *Cassia.*

Taxonomic classification:

Kingdom: Plantae
Sub Division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Sub Class: Rosidae
Order: Fabales
Family: Fabaceae
Genus**:** ***Cassia***

Table: 1
Review of the various medicinal uses of the study plants

Sl No.	Species	Common Name	Uses	References
1	*Cassia alata* Linn. (Shrub)	Candle Bush	Leaves-laxative properties, tea-constipation, intestinal worms, cure eczema, ringworm, white spot fungal skin infections, has wound healing properties, contains chrysophanic acid- ingredient in soaps, shampoos and lotions.	**Pieme, CA et al, 2006, Palanichamy, S, 1990, Ajose, FOA, Villasenor IM, 2002.**
2	*Cassia surratensis* Lam. (Shrub)	Glaucous Cassia	Anti-cancer, anti-inflammatory,,anti-microbial and antioxidant.	**Yik Ling Chew et al (2011)**
3	*Cassia occidentalis* Linn. (Shrub)	Coffee senna/ Negro cassia	Leaves-externally and internally used-skin diseases, itches, scabies, ringworm. Hot decoction preferred to quinine for its tonic properties. Paste of leaves and calcium hydroxide applied to abscesses for quick opening and clearing of pus.	**Ignacimuthu et al (2006), Mugisha MK et** al (2005)

4	*Cassia auriculata* Linn. (Shrub)	Tanner's Cassia	Flowers crushed and taken with goats milk to prevent white discharge in women. Root powder acts as a coagulant, prevents diarrhea, dysentery. Fruit juice –indigestion. It gives relief against skin ailments.	**Yoganarasimhan, 2003. Rastogi R P (1990).**
5	*Cassia sericea* Sw. (Shrub)	One leaf senna	Decoction of vegetables and mature leaves-laxative, useful in curing ring-worm and skin diseases. Plant promising in suppressing the growth of Parthenium.	**A H Rajasab (2004)**
6	*Cassia tora* Linn. (Shrub)	Foetid Cassia	Whole plant-reclamation of saline, alkaline and brackish soils. Seeds are roasted, boiled –used as tea, tones heart muscles and purify blood. Decoction-anti obesity, analgesic, antipyretic, antifungal, antihelmintic, diuretic, expectorant etc. Plants are used to eradicate weeds. Seeds yield yellow, brown and red dye.	**Abdul Rehman (1996), Kirtikar K R et al (1975), Perry L M (1980).**

MATERIALS AND METHODS

I. ***a. Sample collection, Extraction and Processing****:*

Dried plant material was used as a source for the extraction of secondary metabolites in plants.

Fresh plant parts were collected randomly from Bangalore region, India in the year 2011(Sep-Nov). The details of the plant/plant parts screened, their vernacular names and family are presented in table: 1 . All the five plant samples were authenticated by the senior scientists, Department of CES (Centre for Ecological Studies), IISc, Bangalore and plantation officer, Lalbagh Botanical Gardens, Bangalore.

Fresh plant material (leaf) of the Cassia species were separated, washed with tap water, rinsed with distilled water and air dried. The dried leaf of each plant was pulverized using a sterile electric blender, to a fine powder and stored in airtight dark bottles at room temperature. The aqueous extract of the plant samples were prepared by soaking 100gms of dry powdered samples in 200ml of different solvents:

a) Ethanol
b) Methanol
c) Ethyl acetate

for 12 hours. The extracts were filtered using Whatman filter paper No. 42(125mm) and the filtered extracts were stored in airtight dark bottles at room temperature for phyto-chemical analysis.

b. ***Phytochemical screening***:

The different qualitative chemical tests were carried out on the aqueous extract using standard procedures to identify the constituents as described by **Sofawara (1993), Trease** and **Evans (1989), Harborne (1973)** and **Edeoga (2005).**

Qualitative analysis on phytochemical constituents:

Alkaloids: 1ml of the filtrate with 2ml of Drangendroff's reagent shows turbid orange colour.

Tannins: 1ml of filtrate with 2ml of Ferric chloride gives dark green colour.

Saponins**:** 1ml of filtrate with 2ml distilled water, is shaken vigorously and allowed to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 10 minutes indicates the presence of saponins

Anthraquinones: To 1ml of the filtrate add 10ml benzene, filter and add 5ml of 10% (v/v) ammonia to the filtrate and shake well. Development of pinkish coloured solution indicats the presence of anthraquinones.

Anthocyanides: Add 1ml of filtrate with 5ml of dilute HCl, the appearance of pale pink colour indicates the presence of the above compound. .

Phenolic flavonoids: 1ml of filtrate with 2ml of 10% lead acetate gives brown precipitate.

Flavonoids: 1ml of filtrate with 2ml of dilute NaOH shows development of golden yellow colour.

Carbohydrates**:** a.Take1ml of the filtrate with 5ml Benedict's reagent and boil for 5 minutes. Bluish green colour indicates the presence of carbohydrates.

b. To 1ml of filtrate add few drops of Molisch's reagent and few drops of conc. H_2SO_4, which gives purple colour.

c. To 1ml of filtrate add few drops of Fehling's 'A' which gives green colouration.

d.To 1ml of filtrate add few drops of Fehlings'B' which gives Brown colouration.

Proteins: 1ml of filtrate with 5 to 6 drops of Millon's reagent develops white precipitate which turns red on heating.

Steroids*:* To 1ml of the filtrate add 10ml chloroform and 10ml of H_2SO_4 slowly by the sides of the test tube. Upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.

Terpenoids: Take 1ml of the filtrate with 2ml $CHCl_3$ and carefully add few drops of conc H_2SO_4. An interface with a reddish brown colouration is formed.

Cardiac glycosides: To 1ml of the filtrate add 1ml of $FeCl_3$ reagent (mixture of 1 vol of 5% $FeCl_3$ solution + 99 vol of glacial acetic acid) and a few drops of conc H_2SO_4. Greenish blue colour appears within few minutes.

Phlobatannins: To 1ml of the filtrate add few drops of 1% aqueous HCl. A Red precipitate is formed.

Table-2

Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of Cassia alata.

Sl No	Phytochemical Test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract
1	**Test For Alkaloids**	++	++	++
	Dragendorff's Test			
2	**Test for Tannin**	++	++	+
3	**Test for Saponin**	-	-	-
4	**Test for Anthraquinone**	+	-	-
5	**Test for Anthocyanosides**	+	-	-
6	**Test for Phenolic flavonoids**	-	-	-
7	**Test for Flavonoids**	+	+	++
8	**Test for Carbohydrates**			
	Benedict's Test	++	++	++
	Molisch's Test	++	++	++
	Fehling's Test	++	++	+
9	**Test for Proteins**	++	++	+ +
10	**Test for Steroids**	++	++	++
11	**Test for Terpenoids**	++	++	++
12	**Test for Cardiac glycosides**	++	++	++
13	**Test for Phlobatannins**	++	++	+
	Present ++ Moderately present + Absent -			

Table-3

Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of Cassia surratensis.

Sl No	Phytochemical Test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract
1	**Test For Alkaloids**	+	++	++
	Dragendorff's Test			
2	**Test for Tannin**	+	++	+
3	**Test for Saponin**	-	-	-
4	**Test for Anthraquinone**	-	+	-
5	**Test for Anthocyanosides**	-	-	-
6	**Test for Phenolic flavonoids**	-	-	-
7	**Test for Flavonoids**	-	+	+
8	**Test for Carbohydrates**			
	Benedict's Test	++	++	++
	Molisch's Test	++	++	++
	Fehling's Test	++	++	+
9	**Test for Proteins**	++	++	++
10	**Test for Steroids**	+	+	+
11	**Test for Terpenoids**	+	+	+
12	**Test for Cardiac glycosides**	++	++	++
13	**Test for Phlobatannins**	+	+	-
	Present ++ Moderately present + Absent -			

Table-4

Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of Cassia occidentalis.

Sl No	Phytochemical Test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract
1	**Test For Alkaloids**	+	+	++
	Dragendorff's Test			
2	**Test for Tannin**	+	+	+
3	**Test for Saponin**	-	-	-
4	**Test for Anthraquinone**	-	+	-
5	**Test for Anthocyanosides**	-	+	-
6	**Test for Phenolic flavonoids**	+	-	-
7	**Test for Flavonoids**	+	++	-
8	**Test for Carbohydrates**			
	Benedict's Test	++	++	++
	Molisch's Test	++	++	+
	Fehling's Test	++	++	-
9	**Test for Proteins**	++	+	-
10	**Test for Steroids**	+	+	++
11	**Test for Terpenoids**	++	++	+
12	**Test for Cardiac glycosides**	++	++	++
13	**Test for Phlobatannins**	+	++	+
	Present ++ Moderately present + Absent -			

Table-5

Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of Cassia auriculata.

Sl No	Phytochemical Test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract
1	**Test For Alkaloids**			
	Dragendorff's Test	++	++	++
2	**Test for Tannin**	++	++	+
3	**Test for Saponin**	-	-	-
4	**Test for Anthraquinone**	-	-	-
5	**Test for Anthocyanosides**	-	-	-
6	**Test for Phenolic flavonoids**	+	-	-
7	**Test for Flavonoids**	+	+	++
8	**Test for Carbohydrates**			
	Benedict's Test	++	++	++
	Molisch's Test	++	++	++
	Fehling's Test	++	++	+
9	**Test for Proteins**	++	++	++
10	**Test for Steroids**	+	+	+
11	**Test for Terpenoids**	+	+	+
12	**Test for Cardiac glycosides**	++	+	+
13	**Test for Phlobatannins**	+	+	=
	Present ++ Moderately present + Absent -			

Table-6

Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of Cassia sericea.

Sl No	Phytochemical Test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract
1	**Test For Alkaloids**			
	Dragendorff's Test	+	++	++
2	**Test for Tannin**	++	++	-
3	**Test for Saponin**	-	+	-
4	**Test for Anthraquinone**	-	-	-
5	**Test for Anthocyanosides**	-	-	-
6	**Test for Phenolic flavonoids**	+	++	-
7	**Test for Flavonoids**	+	+	-
8	**Test for Carbohydrates**			
	Benedict's Test	++	++	++
	Molisch's Test	++	++	++
	Fehling's Test	++	++	++
9	**Test for Proteins**	++	++	+
10	**Test for Steroids**	++	++	++
11	**Test for Terpenoids**	++	++	-
12	**Test for Cardiac glycosides**	++	++	++
13	**Test for Phlobatannins**	+	++	-
Present ++ Moderately present + Absent -				

Table-7

Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of Cassia tora

Sl No	Phytochemical Test	Ethanol Extract	Methanol Extract	Ethyl acetate Extract
1	**Test For Alkaloids**			
	Dragendorff's Test	++	+	++
2	**Test for Tannin**	++	++	-
3	**Test for Saponin**	-	-	-
4	**Test for Anthraquinone**	-	-	-
5	**Test for Anthocyanosides**	-	-	-
6	**Test for Phenolic flavonoids**	-	-	-
7	**Test for Flavonoids**	-	-	+
8	**Test for Carbohydrates**			
	Benedict's Test	++	++	++
	Molisch's Test	++	++	++
	Fehling's Test	++	++	+
9	**Test for Proteins**	++	++	+
10	**Test for Steroids**	+	+	++
11	**Test for Terpenoids**	+	++	++
12	**Test for Cardiac glycosides**	+	++	++
13	**Test for Phlobatannins**	+	++	-
	Present ++ Moderately present + Absent -			

RESULTS AND DISCUSSIONS

Screening of phytoconstituents from all the six species of *Cassia* leaf extract shows the following.(Table:2,3,4,5,6 & 7).

Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase (**Bonjean K, De Pauw-Gillet M-C, et al.,1998**).

Alkaloids are present in higher quantities in ethyl-acetate extracts of all the six species- *Cassia alata, Cassia surratensis, Cassia occidentalis, Cassia sericea, Cassia tora, Cassia auriculata* only methanol extract of *Cassia alata, Cassia sericea, Cassia auriculata*, only ethanol extract of *Cassia alata, Cassia auriculata* and *Cassia tora*. It is moderately present in methanol extracts of *Cassia occidentalis* and *Cassia tora*; whereas in *Cassia surratensis Cassia occidentalis* and *Cassia sericea* it shows moderate amounts in ethanol extracts.

Tannins reduce the risk of coronary heart diseases (**Janaky Ranjithkumar et al., 2010**). It is present in high quantities in ethanol extracts of *Cassia auriculata, Cassia sericea, Cassia tora* and methyl extract of *Cassia alata, surratensis, Cassia auriculata, Cassia sericea* and *Cassia tora.*

Whereas it is found in moderate amounts in *ethanol extract of Cassia surratensis, Cassia occidentalis*, in methanol extract of *Cassia* occidentalis and ethyl acetate extract of *Cassia surratensis, Cassia occidentalis* and *Cassia auriculata.*

Saponins, present in plants, have been suggested as possible anti-carcinogens. The proposed mechanisms of anti-carcinogenic properties of saponins include direct cytotoxicity, immune modulatory effects, bile-acid binding and normalization of carcinogen-induced cell proliferation. However, the anti-carcinogenic effects of saponins from commonly consumed plant foods have not been studied (**Rao A V, et al 1995**).Soybeans are the most important

sources of dietary saponins and the main protein supplier in many vegetarian diets.

Our results show that saponin is present only in moderate quantities of methanol and ethyl acetate extract of *Cassia sericea* and absent in all the extract of the remaining species.

Anthraquinones are present only in moderate amount in ethanol extract of *Cassia alata* and methyl extract of *Cassia surratensis.*

Anthocyanosides are present in moderate amounts only in methanol extract of *Cassia occidentalis.*

Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (**Singh et al 2007**).Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc (**Ali et al., 2008**). A number of studies have focused on the biological activites of phenolic compounds, which are potential antioxidants and free radical scavengers (**Rice-Evans et al. 1995; Cespedes et al., 2008; Reddy et al., 2008; Chanda and Dave, 2009**).

Phenolic flavonoids are present in high amounts in methanol extract of *Cassia sericea* and in moderate amounts in ethanol extract of *Cassia occidentalis*, *Cassia auriculata* and *Cassia sericea* only.

Flavonoids are also known as vitamin P or plant modifiers, present in high quantities in methanol extract of *Cassia occidentalis* and ethyl acetate extract of *Cassia auriculata.*

Whereas in ethanol extract of *Cassia alata, Cassia auriculata, Cassia sericea* and methanol extract of *Cassia surratensis, Cassia auriculata, Cassia sericea, Cassia alata* and ethyl acetate extract of *Cassia surratensis* and *Cassia tora* it is found in moderate amounts.

Carbohydrate

a. Benedict's test shows that high amounts of carbohydrates in ethanol extract of *Cassia alata, Cassia surratensis, Cassia occidentalis, Cassia auriculata* and *Cassia tora.* It is also seen in high quantities in methanol and ethyl acetate extracts of all six species.

b. Molisch's tests show that high quantities of carbohydrates are present in all the extracts of all the six species taken for study except ethyl acetate extract of *Cassia occidentalis*.

c.In **Fehling's test,** large amounts are seen to be present in ethanol and methanol extracts of all the six species, whereas in ethyl acetate extract it is seen in large quantities only in *Cassia auriculata* and *Cassia sericea,* in moderate amounts in *Cassia surratensis* and *Cassia tora.*

Proteins are present in maximum quantities in all six species of ethanol extract, in methanol extract it is found in all except *Cassia occidentalis*. But in ethyl-acetate extract it is found in *Cassia alata, Cassia surratensis* and *Cassia auriculata*. It is found in moderate quantities in ethyl acetate extract of *Cassia sericea* and *Cassia tora.*

Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents **(Singh AP, 2006**). It is found in large amounts in ethanol and methanol extracts of *Cassia alata* and *Cassia sericea,* ethyl acetate extract of *Cassia occidentalis , Cassia sericea* and *Cassia tora.* It is seen in moderate amounts in Ethanol and methanol extracts of *Cassia surratensis, Cassia occidentalis, Cassia auriculata, Cassia tora,* ethyl acetate extract of *Cassia surratensis* and *Cassia auriculata* only.

Terpenoids are found in maximum amounts in ethanol extracts of *Cassia alata, Cassia occidentalis, Cassia sericea*, methanol extract of the same along with *Cassia tora* and in ethyl acetate extract of *Cassia alata* and *Cassia tora* .It is found to be in moderate amounts in ethanol extract of *Cassia surratensis, Cassia auriculata, Cassia tora*, whereas in methanol extract, only in *Cassia surratensis* and *Cassia auriculataa*, and in ethyl acetate extract of *Cassia surratensis, Cassia auriculata* and *Cassia occiden*talis.

Cardiac glycosides are found in maximum amounts in all except *Cassia tora* of ethanol extract, all except *Cassia auriculata* of methanol and ethyl acetate extract..

Phlobatannins have diuretic property (**Awoyinka OA, et al , 2007**). These are in high amounts in ethanol extract of *Cassia*

alata, methanol extract of *Cassia alata, Cassia occidentalis, Cassia sericea* and *Cassia tora*. It is present in moderate portions in *Cassia surratensis, Cassia occidentalis, Cassia auriculata, Cassia sericea, Cassia tora* and ethanol extract of *Cassia surratensis* and *Cassia* auriculata of methanol extract and in ethyl acetate extract of *Cassia alata* and *Cassia occidentalis*.

CONCLUSION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade. (**Patrick Ekong Ebong et al., 2008**). The medicinal value of the plant lies, in some chemical substance that produces a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins. Plants can be used to discover and screen these bioactive natural organic compounds which may be beneficial for the development of new pharmaceuticals that address today's therapeutic needs.

From the present studies, it can be concluded that all six species of *Cassia (Cassia alata, Cassia surratensis, Cassia auriculata, Cassia occidentalis, Cassia sericea and Cassia tora)* along with the already analysed five species of *Cassia(Cassia spectabilis, Cassia siamea, Cassia fistula, Cassia biflora and Cassia hirsuta)*(**Usha Veerachari et al, 2011**) have many beneficial effects with respect to the presence of the above secondary metabolites which are likely to combat many diseases and also boost the immune system.

The previous phytochemical evaluation of the above mentioned five species of *Cassia* also shows more or less similar results with respect to the presence of the chemical constitutents except anthraquinones, anthocyanosides and phenolic flavonoids.

The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future study.

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REFERENCES

1. Abdul Rahman M.D., 1996, Pengenalan den Penggunaan Herba Ulbatan. Orient Press, Sdn.Bhd, Malaysia; pp.25
2. Ahmed,L., Mohammed, Z., Mohammed, F.Screening of some Indian medicinal plants for their antimicrobial properties. J.Ethnopharmacol.1998.62:183-193.
3. Ajose, FOA, Some Nigerian plants of dermatologic importance. Int J Dermatol, 46(1): 48-55.
4. Ali, S.S., N.Kasoju, A. Luthra, A. Singh, H.Sharanabasava, A.Sahuand U. Bora, 2008. Indian medicinal herbs as source of antioxidants. Food. Res. Int., 41:1-15.
5. Anjali D. Ruikar et al, Antimicrobial screening of medicinal plant- Artemisia pallens, Int. J.Pharmtech Res.2009, 1(4).pp1164-1166.
6. Anjali D. Ruikar et al, Antimicrobial screening of medicinal plant- Artemisia pallens, Int. J.Pharmtech Res.2009, 1(4).pp1164-1166.
7. Arshad Hussain et al. Antibacterial activity of the leaves of Coccinia indica (W and A)

W of India. Advances in biological research. 2010.4(5): 241-248.
8. Asolkar, L.V., et al. Second supplement to Glossary of Indian Medicinal plants with active principle part=1 (A-K). Council of Scientific and Industrial research (PID) , New Delhi.1992.217-218.
9. Awoyinka OA, Balogun IO, Ogunnowo AA (2007). Phytochemical screening and in vitro bioactivity of Cnidoscolus aconitifolius (Euphorbiaceae). Journal of Medicinal Plants Research, 1(3): 063-065.
10. Bonjean K, De Pauw-Gillet M-C, et al ., J.Ethnopharmocol 1998, 69,241-246.
11. Cassia alata Linn. Database on important Medicinal and Aromatic plants.
12. Chellaiah Muthu, Savarimuthu, Ignacimuthu, Medicinal plants used by Traditional healers in Kancheepuram District of TamilNadu, India. 2006-Journal of Ethnobiology and Ethnomedicine.
13. Edeoga, H.O., Okwu, D.E., Mbabie, B.O.(2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4:685-688.
14. Harbone, J.B.(1973). Phytochemical Methods, Chapman and Hall, Ltd., London, p49-188.
15. Harbone, J.B.1998. Phytochemical Methods 3rd Edn., Chapman and Hall, London, ISBN:0-412-57260-5,pp:1-302.
16. Janaky Ranjithkumar et al, 2010, Secondary metabolite investigation, J.Chem.Pharm.Res., 2(4):371-377.
17. Jayyed Press, New Delhi: 30-45.
18. Kirtikar, K.R and Basu, B.D.1975. Indian Medicinal Plants, Volume II Second Edition.
19. Mugisha MK, Origa, HO, Traditional herbal remedies used in management of sexual impotence and erectile dysfunction in Western Uganda, African Health Sciences, 5(1), 2005,40-49.
20. Palanichamy, S. And Nagarajan, S. Antifungal activity of Cassia alata leaf extract. J.Ethnopharmacol, 29; 337-340, 1990.
21. Panneerselvam A, A.sivaraj etc, Antibacterial acyivity of Coccinia grandis leaf extract on selective bacterial strains, Journal of Applied Pharmaceutical Science 01(07); 2011:120-123.
22. Parsons, W.T. Cuthbertson, E.G., 1992, Noxious weeds of Australia. In data Press, Melbourne: 455-456.
23. Patrick Ekong et al., The Antidiabetic Efficacy of Combined Extracts From Two Continental Plants: Azadirachta indica (A.Juss) (Neem) and Vernonia amygdalina (Del:) (African Bitter Leaf). Am. J. Biochem. Biotech. 2008.4(3):239-244.
24. Perry, L.M.1980 Medicinal plants of East and Southeast Asia, attributed properties and uses.MIT Press, Cambridge, Mass, U.S.A.
25. Pieme, CA, Penlap, VN, Nkegoum, B, Taziebou, CL, Tekwu, EM, Etoa FX, Ngongang, J. Evaluation of actue and subacute toxicities of aqueous ethanolic extract of leaves of Senna alata (L.)Roxb (Caesalpiniaceae). African I Biotech 5(3); 283-289, 2006.
26. Rajasab, A.H.2004, Indian Journal of Traditional Knowledge., vol.3(4). Mopr.niscair.res.in
27. Rao AV, Sung MK. Saponins as anticarcinogens. J Nutr (1995) 125:717S-24S.
28. Rastogi RP, Mehrotra BN; Compendium of Indian Medicinal plants. Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, India, 1990-1994, I-V.
29. Rice-Evans, C., N.J.Miller, G.P. Bolwell, P.M.Bramley and J.B.Pridham, 1995. The relative anioxidants activities of plant-derived polyphenolic flavonoids. Free Rad.Res.,22: 375-383.Sofowara A (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria.p.289.
30. Singh AP(2006). Short Review: Distribution of steroid like compounds in plant flora. Pharmacognosy Magazine, 2(6):87-89.

31. Singh, R., S.Singh et al 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of Acacia auricliformis A. Cunn. Food Chem. Toxicol., 45:1216-1223.
32. Trease GE, Evans WC (1989). Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.
33. Usha Veerachari et al, 2011, Preliminary phyto-chemical evaluation of the leaf extract of five *Cassia* species, J.Chem. Pharm. Res., 3(5);574-583.
34. Villasenor IM, Canlas AP, Pascua MPI, Sabando MN, Solivan, LA. Bioactivity studies on studies on Cassia alata.Jinn. leaf extract. Phytother Res. 16:93-96,2002.
35. Yoga Narasimhan SN, Medicinal plants of India, 2003,II.