Int J Pharm Bio Sci 2015 April; 6(2): (B) 1062 - 1070

Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYTOCHEMICAL CHARACTERIZATION OF TWELVE MEDICINAL PLANTS USED FOR SICKLE CELL DISEASE MANAGEMENT IN CHHATTISGARH

AKRITI GUPTA, SHUBHANGI SHARMA, HENU KUMAR VERMA, DIVYA SUDHIR TYAGI, PANKAJ KISHOR MISHRA* AND P K PATRA

Medical Biotechnology, Department of Biochemistry, Pt. J. N. M. Medical College, Raipur-492001, India

ABSTRACT

Medicinal plants can be a source of succour in the control of sickle cell disease (SCD) in Chhattisgarh. The lower strata of the state rely heavily on traditional medicine due to their cultural alignment as well as their inability to afford the cost of treatment offered by orthodox medical practitioners. Twelve plant species have been selected to examine their phytochemical constituents from SCD prone areas of state currently used to the management of disease. Qualitative analysis of phytochemical constituents' viz. tannins, flavonoids, terpenoids, saponins, phenol, steroids, phlobatannins, carbohydrates, glycosides, coumarins, alkaloids, proteins, emodins, anthraquinones, anthocyanins, leucoanthocyanins and quantitative analysis of alkaloids, tannin, saponins, flavonoids and total phenol was performed by the standard protocol available in the literature. Quantitative analysis of alkaloid, tannin, saponin, flavonoids and total phenol had revealed that *Momordica charantia* possessed maximum alkaloid (5.92%w/w) and tannin (9.44%w/w), *Aloe vera* highest saponin (7.15%w/w) & flavonoids (8.23%w/w) and *Allium sativum* highest total phenol (12.43%w/w) content. Present findings will be very compassionate to elaborate our study objectives to investigate better management options and reduce the symptomatic crisis of SCD.

KEYWORDS: Medicinal plants, Phytochemical constituents, Sickle cell disease

PANKAJ KISHOR MISHRA
Medical Biotechnology, Department of Biochemistry, Pt. J. N. M. Medical College, Raipur-492001, India

***Corresponding author**

INTRODUCTION

Sickle-cell disease (SCD), or sickle-cell anemia is an autosomal recessive genetic blood disorder with over dominance, characterized by a banana, crescent-moon or sickle shaped red blood cells that assume an abnormal, rigid and known to be one of the diseases afflicting the population living mostly in Africa, South America and Asia. In Chhattisgarh (India) on the basis of data shared by an ongoing sickle-cell project at Department of Biochemistry, Pt. J.N.M. Medical College, Raipur, approximately 10% population has prevalence of SCD which is tending to become a public health problem. According to 2011 census around 25 lakhs human beings were affected from SCD in Chhattisgarh state only. Majority of these was identified as carriers by doing the screening of 10.52 lakhs children from entire state. Several therapies and chemical substances investigated only for management because no specific drugs are yet available for permanent cure. Potential agents employed for inhibition of hemoglobin S polymerization include hydroxyurea, piracetam, calcium antagonists in order to increase the fetal hemoglobin rate (HbF) and may be toxic especially for a long time of use[1-2]. This study has been on-going to determine the therapeutic potential of natural products such as medicinal plants, nutritional complement against sickle cell disorder. Although, some researchers are not in favor of use of natural supplements in the management of sickle cell disorder[3] but that as it may many SCD patients are using several natural products obtained from plants available in their local territories for effective management of erythrocytes sickling in Chhattisgarh. Twelve medicinal plants were preferred in this study for phytochemical characterization is of great importance to the health of SCD individuals. Many of these indigenous medicinal plants are used as spices and vegetables. *Aloe vera* L. (Xanthorrhoeaceae) is one of the most commonly used herbs in herpes, constipation, diabetes, psoriasis, as anti-inflammatory agent and to mitigate the effect of alcohol-induced liver damage[4]. *Allium sativum* L. (Ameryllidaceae) has been widely recognized as a valuable spice and its principal medicinal uses are to lower blood pressure and cholesterol, fight infections, and prevent cancer[5]. *Anacardium occidentale* L. (Anacardiaceae) leaves, stem and bark extracts are utilized widely for the treatment of diarrhea, dysentery and colonic pain. It has also been reported to possess antidiabetic, antioxidant, antimicrobial, anti-inflammatory and anti-ulcerogenic activity[6]. *Carica papaya* L. (Caricaceae) is a lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. It can be used for treatment of a numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many, as a Neutraceutical[7]. *Cajanus cajan* L. (Fabaceae) is the most important grain legume crop of rain-fed agriculture in semi-arid tropics with high levels of proteins and important amino acids like methionine, lysine and tryptophan. Cajachalcone, 2′,6′-dihydroxy-4-methoxy chalcone, has been isolated as the biologically active constituent could be used for anti-malarial drug discovery[8.] *Camellia sinensis* L. (Theaceae) is commonly known as green tea in the India having various important therapeutic potentials such as anticancer activity, lipid lowering activity, immunomodulatory effect, antiviral activity, antibacterial activity, antispasmodic, anticataract, antioxidant, antidiabetic and antigenotoxic effect[9]. *Eugenia caryophyllata* L. (Myrtaceae) used as a spice in India and has been traditionally in the treatment of roundworms and tapeworms, asthma, toothache, sore throat, dental, respiratory disorders, digestive system ailments, dyspepsia, gastritis, diarrhea, as antipyretic, aphrodisiac, carminative, appetizer, expectorant, antiemetic, anxiolytic, myorelaxant, analgesic, decongestant, anti-inflammatory, hypnotic, vermifuge, antibacterial agent etc[10]. *Momordica charantia* L. (Cucurbitaceae) is a tropical vegetable in Indian cusine and has been used extensibly as a

remedy for diabetes, cancer along with antibacterial activity, antiviral activity, antiHIV activity and acting as a antifertility agents[11]. *Pisidium guajava* L. (Myrtaceae) is a well known traditional medicinal plant used in various indigenous systems of medicine and widely distributed throughout India. Tranquilizing effect of *P. guajava* on intestinal smooth muscle have been investigated inhibiting chemical processes found in diarrhea and aid in the re-absorption of water in intestines through the inhibition of intracellular calcium release[12]. *Picrorhiza kurroa* (Plantaginaceaea) contains a 'bitter principle' which is a mixture of two molecules, the irioid glycosides known as picroside I and picroside II. Medicinally used for liver function alteration, and also extend to states of intrinsic liver dysfunction (viral hepatitis and NAFLD from a high fat diet)[13]. *Piper guineense* (Piperaceae) is used as spice and the richest source of a wide range of natural products including volatiles oils, lignans, amides, flavonoids and polyphenols. Leaves are used for respiratory infections and for female infertility while its fruits are used as an aphrodisiac[14]. *Sorghum bicolor* L. (Poaceae) is a staple cereal in hot dry tropics, the threshed grain ground into wholesome flour possessing anti-anemic and antimicrobial properties[15]. Plant products have been part of phytomedicines which can be derived from all parts of the plant like bark, leaves, flowers, fruits, seeds, etc. since time immemorial. This study includes phytochemical screening of 12 medicinal plants was reported in view of proposing an effective herbal recipe for the management of SCD.

MATERIALS AND METHODS

1. Collection of plant materials

Twelve medicinal plants were collected locally from the farm lands of sickle cell prone area of Chhattisgarh in the month of September, 2014. The specimens were authenticated by renowned taxonomist before starting phytochemical characterization. Plant species selected during present investigation were given in Table 1. Plant parts were washed, cleaned and chopped into pieces and dried at 40^{o}C in thermostatically controlled oven until they attained a constant weight. The samples were then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

2. Extraction

Water extracts were prepared by soaking 10 g each of the dry powdered plant materials in 100 ml of distilled water for 72 hours at room temperature with continuous stirring. After 72 hours the extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. Filtrate was centrifuged at 2500xg for 15 minutes and supernatant was stored in sterile bottles at 5°C for further phytochemical analysis.

3. Preliminary Phytochemical Screening

Preliminary screening of the above twelve extracts for various phytochemical constituents was carried out using standard procedures[16] as described in Table 2.

4. Quantitative Determination of Phytochemical Constituents

(i) Alkaloid

Five gram of the sample was weighed into a 250 ml beaker, 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated NH_4OH was mixed dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed[16].

(ii) Tannin

Five hundred milligram of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the

filtered sample was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min[17].

(iii) Saponin

Twenty gram of each sample was placed into a conical flask and 100 ml of 20% aqueous ethanol was added to the plant sample. The samples were heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The collective residues were reduced to 40 ml over a hotwater bath. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded and the process of purification was repeated. 60 ml of n-butanol was added and combined n-butanol extracts were washed twice with 10 ml of 5% NaCl. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight[18].

(iv) Flavonoids

Ten gram of each plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 41. The filtrate was allowed to be evaporated into dryness over a water bath and weighed to a constant weight[19].

(v) Total phenol

Total phenolic content of the methanolic extract of all selected plants was determined by modified method of Makkar et al[20], using tannic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 600 µg of tannic acid/ml. 250 µl of diluted extract or tannic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl FCR. The samples were mixed well and then allowed to for 5 min at room temperature in order to allow complete reaction with the FCR. 2.5 ml of 7% Na_2CO_3 was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue color solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min.

RESULTS AND DISCUSSION

1. Qualitative analysis of selected species

Preliminary phytochemical screening of aqueous extracts of different parts of twelve medicinal plant species has been carried out by following the methods reported in literature Table 2. Recorded observations revealed the presence of a wide range of phytoconstituents including tannins, flavonoids, terpenoids, saponins, phenol, steroids, phlobatannins, carbohydrates, glycosides, coumarins, alkaloids, proteins, emodins, anthraquinones, anthocyanins and leucoanthocyanins. The results investigated have been summarized in Table 3 and 4 showed the presence of medicinal activity as well as exhibiting physiological activity[21].Tannins were found in 6 medicinal plants out of 12 selected contributes property of astringency i.e. faster the healing of wounds and inflamed mucous membrane[22]. Terpenoids were present in 7 medicinal plants attributed for analgesic and anti-inflammatory activities. It has been examined that *A sativum, C papaya, E caryophyllata, P guajava, P kurroa and P guineense* contained steroidal compounds are of great significance and awareness in pharmacy due to their relationship with sex hormones and are used by expectant mothers or breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones[23]. Phlobatannins are present in *A occidentale, E caryophyllata, P guajava* and *P guineense*. Phlobatannins have been reported for its wound healing properties, these are anti-inflammatory, analgesic and antioxidant[24]. Coumarins were present in all aqueous extract except *P guajava* which is a potent antioxidant due to its ability to scavenge free radicals and to chelate metal ions[25]. Anthocyanins were present in *A sativum, C papaya, E*

caryophyllata, P kurroa and P guineense, provides strength to human immune system to work more efficiently to protect against viral infections[26]. Leucoanthocyanine substances were found in *A sativum, C papaya and P kurroa* which acts as a colorless precursor for anthocyanin. Emodin compounds were present only in *C papaya and C sinensis*. Several pharmaceutical studies have demonstrated biological effects of emodin as anticancer, antimicrobial and anti-inflammatory effects[27].

2. Quantitative analysis of selected species

The quantitative (%w/w) determination of alkaloid, tannin, saponin, flavonoids and total phenol of different plant species has been undertaken as per standard procedures and the results have been reported in Table 5. *M charantia* has been found to posses 5.92 %w/w alkaloid content followed by *C papaya*, *A sativum*, *P kurroa, C sinensis*, *C cajan* and *S bicolor.* The use of alkaloid contains plants as dyes, spices, drugs or poisons can be traced long back and well known for their CNS activities[28]. Tannin content was examined maximum in *M charantia* 9.44%w/w and minimum in *P guineense* 4.43%w/w while *A sativum, A occidentale*, *C papaya, C sinensis, E caryophyllata P guajava* were totally devoid of them. Tannins generally preferred for the treatment of inflammation, leucorrhoea, gonorrhea, burn, piles, diarrhea and as antidote in the treatment of alcaloidal poisoning[29]. Total saponin content was found to be highest 7.15%w/w in *A vera* and minimum 4.62%w/w in *M charantia* showed antibiotic, antifungal, antiviral, hepatoprotective anti-inflammatory and anti-ulcer activities[30]. *A vera also* showed maximum flavonoids 8.23%w/w and was found to be minimum 4.86%w/w in *C sinensis.* They are consumed in the form of fruits and vegetables are non-toxic as well as potentially beneficial to the human body; up till now, more than 2000 different flavonoids have been isolated from vegetables[31]. Among the samples analyzed, *A sativum* was found to contain maximum 12.43%w/w followed by 7.88%w/w and 6.55%w/w in *A occidentale* and *C papaya* phenolics respectively. Phenolic compounds can be suggested significantly contributed to the antioxidant potential of selected plant species[32].

Table1

Ethnobotanical information of selected medicinal plant species for phytochemical characterization in sickle cell prone area of Chhattisgarh

S. No.	Plant Species	Local Name	Part Used
1	*Aloe vera* L.	Aloe vera	Leaf
2	*Allium sativum* L.	Garlic	Bulb extract
3	*Anacardium occidentale* L.	Cashew	Leaves
4	*Carica papaya* L.	Papaya	Fruit
5	*Cajanus cajan* L.	Pigeon pea	Seed
6	*Camellia sinensis* L.	Green tea	Leaves buds
7	*Eugenia caryophyllata* L.	Clove	Flower (whole clove)
8	*Momordica charantia* L.	Bitter melon	Seed
9	*Pisidium guajava* L.	Apple guavava	Leaves
10	*Picrorhiza kurroa*	Kutki	Rhizome
11	*Piper guineense*	Black peper	Seed ,dried fruit
12	*Sorghum bicolor* L.	Milo	Grains

Table 2
Preliminary phytochemical tests for plant extracts

Phytoconstituents	Test	Observation
Tannins **(Braymer's Test)**	2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate
Flavonoids	1ml extract + 1ml $Pb(OAc)_4$ (10%)	Yellow coloration
Terpenoids	2ml extract + 2ml $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration
Saponins **(Foam Test)**	(a) 5ml extract + 5ml H_2O + heat (b) 5ml extract + Olive oil (few drops)	Froth appears Emulsion forms
Phenol	4ml extract + 2ml ethanol + 2-3 drops $FeCl_3$ (5%)	Red coloration
Steroids **(Salkowski Test)**	2ml extract + 2ml $CHCl_3$ + 2ml H_2SO_4 (conc.)	Reddish brown ring at junction
Phlobatannins **(Precipitate Test)**	2ml extract + 2ml HCl (1%) + heat	Red precipitate
Carbohydrates **(Molisch's Test)**	2ml extract + 10ml H_2O + 2 drops Ethanolic α-naphthol (20%) + 2ml H_2SO_4(conc.)	Reddish violet ring at the junction
Glycosides **(Liebermann's Test)**	2ml extract + 2ml $CHCl_3$ + 2ml CH_3COOH	Violet to blue to green coloration
Coumarins	2ml extract + 3ml NaOH (10%)	Yellow coloration
Alkaloids **(Hager's Test)**	2ml extract + few drops of Hager's reagent	Yellow precipitate
Proteins **(Xanthoproteic Test)**	1ml extract + 1ml H_2SO_4(conc.)	White precipitate
Emodins	2ml extract + 2ml NH_4OH + 3ml Benzene	Red coloration
Anthraquinones **(Borntrager's Test)**	3ml extract + 3ml Benzene + 5ml NH_3(10%)	Pink, Violet or Red coloration in ammonical layer
Anthocyanins	2ml extract + 2ml HCl (2N) + NH_3	Pinkish red to bluish violet coloration
Leucoanthocyanins turns	5ml extract + 5ml Isoamyl alcohol	Organic layer into Red

Table 3
Results of preliminary phytochemical screening of the selected medicinal plants

Variable	*A vera*	*A sativum*	*A occidentale*	*C papaya*	*C cajan*	*C sinensis*
Tannins	+ve	- ve	- ve	- ve	+ ve	- ve
Flavonoids	+ ve	+ ve	- ve	+ ve	- ve	+ ve
Terpenoids	- ve	+ ve	+ ve	+ ve	- ve	- ve
Saponins	+ ve	+ ve	- ve	- ve	+ ve	- ve
Phenol	- ve	+ ve	+ ve	+ ve	- ve	- ve
Steroids	- ve	+ ve	- ve	+ ve	- ve	- ve
Phlobatannins	- ve	- ve	+ ve	- ve	- ve	- ve
Carbohydrates	- ve	- ve	+ ve	+ ve	- ve	- ve
Glycosides	+ ve	- ve	- ve	- ve	- ve	+ ve
Coumarins	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Alkaloids	- ve	+ ve	- ve	+ ve	+ ve	+ ve
Proteins	- ve	- ve	- ve	- ve	- ve	- ve
Emodins	- ve	- ve	- ve	+ ve	- ve	+ ve
Anthraquinones	- ve	- ve	- ve	- ve	- ve	- ve
Anthocyanins	- ve	+ ve	- ve	+ ve	- ve	- ve
Leucoanthocyanins	- ve	+ ve	- ve	+ ve	- ve	- ve

(+)= Presence, (-)= Absence
Note: Each datum is the average of three Independent Determinations

Table 4
Results of preliminary phytochemical screening of the selected medicinal plants

Variable	*E caryophyllata*	*M charantia*	*P guajava*	*P kurroa*	*P guineense*	*S bicolor*
Tannins	- ve	+ ve	- ve	+ ve	+ ve	+ ve
Flavonoids	- ve	- ve	- ve	+ ve	+ ve	- ve
Terpenoids	+ ve	- ve	+ ve	+ ve	+ ve	- ve
Saponins	- ve	+ ve	+ ve	- ve	- ve	- ve
Phenol	- ve	- ve	- ve	- ve	- ve	- ve
Steroids	+ ve	- ve	+ ve	+ ve	+ ve	- ve
Phlobatannins	+ ve	- ve	+ ve	- ve	+ ve	- ve
Carbohydrates	+ ve	- ve	+ ve	- ve	- ve	- ve
Glycosides	- ve	- ve	- ve	- ve	- ve	- ve
Coumarins	+ ve	+ ve	- ve	+ ve	+ ve	+ ve
Alkaloids	- ve	+ ve	- ve	+ ve	- ve	+ ve
Proteins	- ve	- ve	- ve	- ve	- ve	- ve
Emodins	- ve	- ve	- ve	- ve	- ve	- ve
Anthraquinones	- ve	- ve	- ve	- ve	- ve	- ve
Anthocyanins	+ ve	- ve	- ve	+ ve	+ ve	- ve
Leucoanthocyanins	- ve	- ve	- ve	+ ve	- ve	- ve

(+)= Presence, (-)= Absence
Note: Each datum is the average of three Independent Determinations

Table 5
Quantitative estimation of alkaloids, tannin, Saponins, flavonoids and total phenol

Plant species	**Alkaloid** (%w/w)	**Tannin** (% w/w)	**Saponin** (% w/w)	**Flavonoids** (%w/w)	**Total phenol** (%w/w)
Aloe vera L.	-	8.65	7.15	8.23	-
Allium sativum L.	5.32	-	6.59	7.34	12.43
Anacardium occidentale L.	-	-	-	-	7.88
Carica papaya L.	5.40	-	-	6.21	6.55
Cajanus cajan L.	4.54	5.12	5.80	-	-
Camellia sinensis L.	4.68	-	-	4.86	-
Eugenia caryophyllata L.	-	-	-	-	-
Momordica charantia L.	5.92	9.44	4.62	-	-
Pisidium guajava L.	-	-	4.98	-	-
Picrorhiza kurroa	4.86	5.78	-	6.11	-
Piper guineense	-	4.43	-	8.22	-
Sorghum bicolor L.	4.26	6.31	-	-	-

Note: Each datum is the average of three Independent Determinations

CONCLUSION

Due to the challenges faced by scientists in developing countries, so many sources of constituents capable of ameliorating the sickle cell crises have been investigated with a view to contributing to the search for substances that would be effective in solving the SCD problem. In Chhattisgarh medicinal plants have been used by patients in the treatment of painful crises associated with SCD especially among the lower socio-economic class who cannot afford the high cost of western medicine as well as traditionalists who simply believe in their efficacy. One of our primary sources of information based on phytochemical screening of twelve local medicinal plants whose medicinal knowledge was passed to them by their ancestors. Scientific evaluations will be undertaken by our research team to authenticate the traditional use of these plants as antisickling agents with a potential for use in the clinical management by conducting an advance study of various metabolic and signaling pathways *in vitro* and *in vivo*.

REFERENCES

1. Mpiana P.T, Mudogo V, Tshibangu D.S.T, Kitwa E.K, Kanangila A.B, Lumbu J.B.S, Ngbolua K.N, Atibu E.K and Kakule M.K, Antisickling activity of anthocyanins from Bombax pentadrum, Ficus capensisandZiziphus mucronata: Photodegradation effect, J Ethnopharmacol, 120(3): 413–8, (2008).
2. Ibraheem N.K, Ahmed J.H and Hassan M.K, The effect of fixed oil and water extracts of Nigella sativaon sickle cells: an in vitro study, Singap Med J, 51(3): 230–4, (2010).
3. Imaga N.O.A, The use of phytomedicines as effective therapeutic agentsin sickle cell anemia, Scientific Res Essays, 5(24): 3803–7, (2010).
4. Boudreau M.D, Beland F.A, An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller), *Aloe vera*, J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 24(1):103-54, (2006).
5. Londhe V.P, Gavasane A.T, Nipate S.S, Bandawane D.D and Chaudhari P.D, Role of garlic (*Allium sativum*) in various diseases: an overview, J Pharma Res Opinion,1(4): 129-34, (2011).
6. Kumar V, Doss A and Thangavael K.P, Antioxidant and antimicrobial activity using different extracts of *Anacardium occidentale* L, Int J App Biol Pharma Tech, 2(3): 436-43, (2011).
7. Aravind D, Debjit B, Duraivel S and Harish G, Traditional and medicinal use of *Carica papaya*, J Med Plants Studies, 1(1): 7-15, (2013).
8. Ajaiyeoba E.O, Ogbole O.O, Abiodun O.O, Ashidi J.S, Houghton P.J and Wright C.W. Cajachalcone: An Antimalarial Compound from *Cajanus cajan* Leaf Extract, J Parasit Res, http://dx.doi.org/10.1155/2013/703781, (2013).
9. Bhatt P.R, Pandya K.B and Sheth N.R. *Camellia sinensis* (L.): The medicinal beverage: a review, Int J Pharma Sci Rev Res, 3(2): 6-9, (2010).
10. Singh J, Baghotia A and Goel S.P. Eugenia caryophyllataThunberg (Family Myrtaceae): A Review, Int J Res Pharma Biomed Sci, 4, 1469-75(2012).
11. Kumar D.S, Sharathnath K.V, Yogeswaran P, Harani A, Sudhakar K, Sudha P and Banji D. A medicinal potency of *Momordica charantia*, Int J Pharma Sci Rev Res, 1(2): 95-100, (2010).
12. Belemtougri R.G, Constantin B, Cognard C, Raymond G and Sawadogo L, Effects of two medicinal plants *Psidium guajava* L. (Myrtaceae) and *Diospyros mespiliformis* L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture, J of Zhejiang Univ Sci B, 7(1):56-63, (2006).
13. Upadhyay D, Dash R.P, Anandjiwala S and Nivsarkar M, Comparative pharmacokinetic profiles of picrosides I and II from kutkin, Picrorhiza kurroa extract and its formulation in rats, Fitoterapia , 85, 76-83, (2013).
14. Kamtchouing P, Mbongue G.Y.F, Dimo T, Watcho P, Jatsa H.B and Sokeng S.D, Effects of Aframomum melegueta and Piper guineense on sexual behaviour of male rats, Behav Pharmacol, 13, 243, (2002).
15. Ogwumike O.O, Hemopoietic effect of aqueous extract of the leaf sheath of *Sorghum bicolor* in albino rats, African J Biomed Res, 5, 69-71 (2002).
16. Harborne J.B, Phytochemical Methods, Chapman and Hall Ltd., London; pp.49-188, (1973).
17. Van-Burden T.P, Robinson W.C, Formation of complexes between protein and Tannin acid, J. Agric. Food Chem, 1:77, (1981).
18. Obdoni B.O, Ochuko P.O, Phytochemical studies and comparative efficacy of the crudeextracts of some Homostatic plants in Edo and Delta States of Nigeria, Glob J Pure Appl Sci, 8b, 203-8, (2001).

19. Arumugam P, Ramamurthy P, Santhiya S.T and Ramesh A, Antioxidant activity measured in different solvent fractions obtained from Mentha spicataLinn: An analysis by ABTS. + decolorization assay, Asia Pac J Clin Nutr, 15(1):119-24, (2006).
20. Makkar H, Bluemmel M, Borowy N and Becker K, Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods, J Sci Food Agric, 61, 161-65, (1993).
21. Sofowara A, Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, p.289, (1993).
22. Okwu D.E., Josiahm C, Evaluation of the chemical composition of two Nigerian medicinal plants. Afri J Biotech, 5, 357-361, (2006).
23. Okwu D.E, Evaluation of the chemical composition of indigenous spices and flavouring Agents, Global J Pure Appl Sci, 7(3): 455-59, (2001).
24. Ayinde B.A, Omogbai E.K and Amaechina F.C, Pharmacognosy and hypotensive evaluation of Ficus exasperata Vahl (Moraceae) leaf, Acta Pol Pharm, 64, 543-46, (2007).
25. Tseng A, Chemoprevention of tumors in MTV-H ras transgenic mice with coumarins, Proc Am Assoc Cancer Res, 32, 2257, (1991).
26. Li A.L, Shu S.H, Qin H.L, Lee S.M.Y, Wang Y.T and Du G. H, In vitroanti-influenza viral activities of constituents from *Caesalpinia sappan*, Planta Med, 75, 337-9, (2009).
27. Wang C.H, Gao Z.Q, Ye B, Cai, J.T, Xie C.G, Qian K.D and Du Q, Effect of emodin on pancreatic fibrosis in rats, World J. Gastroenterol, 13, 378-82, (2007).
28. Lewis W.H, Elvin-Lweis P.F, Medical botany: Plants affecting human health, 2nd Edn, John Wiley & Sons, Washington, (2003).
29. Buzzini P, Arapitsas P, Goretti M, Brand E, Turchetti B, Pinelli P, Leri F, and Romani A, Antimicrobial and antiviral activity of hydrolysable tannins, Mini Rev Med Chem, 8, 1179-78, (2008).
30. Zhang Y.W, Due D.Q, Zhang L, Chen Y.J and Yao X.S , Effects of Ginsenosides from Panax ginseng on cell-to-cell communication function mediated by gap junctions, Plants Med, 67, 417-22, (2001).
31. Taiz L, Ziegler E, Plant Physiology, Sinauer Associates, Publishers, Massamchusetts, 4th edn, ch. 13.pp.315-344, (2006).
32. Cai Y, Luo Q, Sun M and Corke H, Antioxidant activity and phenolic compounds of 112 traditional Chinise medicinal plants associated with anticancer, Life Sci, 74, 2157-84, (2004).