Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2018; 10(8): 210-215

ISSN- 0975 1556

Research Article

Qualitative and Quantitative Analytical Studies for the Screening of Phytochemicals from the Leaf Extracts of *Senna alexandrina* Mill

Leelavathi V, Udayasri P

Department of Biotechnology, Acharya NagarjunaUniversity, Guntur

Available Online:25th August, 2018

ABSTRACT

The increasing resistance to the existing synthetic drugs is being substituted with the alternative herbal drugs as an importance source of new agents for treating various ailments. *Senna alexandrina* Mill. is a well known plant in Asian countries including India, which exhibits a wide range of pharmacological activities. It has been used in Indian folk medicine in the form of decoctions and infusions to treat bacterial infections and was also reported to be an effective against a variety of skin diseases. The current investigation was carried out to explore the phytochemical components of the selected plant by performing preliminary biochemical and quantitative analysis by employing UV-visible spectroscopy. The crude extracts were scanned in the UV wavelength ranging from 200-800nm by using Perkin Elmer Spectrophotometer and the results indicate that alkaloids, flavonoids carbohydrates, proteins and saponins are the more prevalent components in the methanolic and ethyl acetate extracts of *Senna alexandrina* leaf.

Keywords: UV-VIS Spectroscopy, Phytochemical screening, Leaf extracts, Pharmacological activities.

INTRODUCTION

Alexandrian senna is native to northern and northeastern Africa, growing wild in semi desert and Sudano - Sahelian zones of Africa, including Egypt, Morocco, Mauritania, Mali, and Sudan. It is cultivated in the valley of the Nile in Sudan, southern China and India[1]. As an annual crop; it remains in field for 110–130 days. The plant bears compound leaves, made up of 5–8 pairs of shortly stalked oval-lanceolate leaflets (2.5cm × 1.5cm) and produce successive flush of flowering shoots both in axillary and sub terminal position 60–70 days after sowing. The flowers are large and brilliant yellow in colour, producing medium-sized pods (3.5cm–6.5cm × 1.5cm) after 90 days. They contain 5–8 yellowish, flat seeds. It is predominantly self-pollinated crop but out crossing could be high (20%) through beetles[2]. Dried fruit (pods) and leaves (Dried leaflets), used in making tablets, the fruit is most commonly used for preparation of senna infusion (tea). People in Northern Africa and South Western Asia have used *Senna* as a laxative for centuries. It was considered as a "cleansing" herb because of its cathartic effect. In addition, the leaves were used for treating Anemia, Anorexia, Biliousness, Bronchosis, Burns, Cancer, Cholera, Constipation, Cramp, Dermatosis, Dysentery, Dyspepsia, Enterosis, Fever, Fungal infections, Gastrosis, Gonorrhea, Gout, Halitosis, Hemorrhoid, Hepatosis, Herpes, Hiccups, Infection, Jaundice, Leprosy, Leukemia, Mycosis, Nausea, Neural disorders, Pimple, Ringworm, Splenosis, Syphilis, Typhoid, Venereal Disease, Viral diseases, antihelmenthic and Wound healing (Duke's Handbook of Medicinal Plants of the Bible). Pharmacological investigations show that sennosides A and B account for the entire activity of the senna leaves and pods. Leaves contain glycosides, sennoside A, B, C and D. Two naphthalene glycosides have been isolated from leaves and pods. The medicinal properties of *Senna* can be attributed mainly to the anthraquinone glycosides, especially sennoside A and B. It appears that the aglycone portion is responsible for its action[1].

In folk medicine Senna was applied for curing different ailments as it shows Anthelmintic, Antidysenteric, Antihepatotoxic, Antiherpetic, Antileukemic, Antispasmodic, Antiviral, Antibacterial (staphylococci and Bacillus Coli)[3]. Antifungal (Microsporumaudouini, etc.). Hepatoprotective and Neuroprotectiveproperties[4]. It was also exhibiting Carminative, Cathartic, Expectorant, Mutagenic, Trypsin Inhibition, Purgative, vermifuge[5], Diuretic, Colon Cleansing and body detoxifing properties[6]. The Selected Medicinal plant *Senna alexandrina* were screened for the bioactive compounds by employing different Biochemical protocols and analytical techniques such as UV-Visible spectrophotometry.

MATERIALS AND METHODS

Plant Materials

The leaf of *Senna alexandrina* was purchased from the local market, it was cleaned and dried under shade and made them into powder and the selected solvents Methanol, Acetone and ethyl acetate were procured from the National scientific products, Guntur and were used for the preparation of plant extracts by Soxhlet method, the extract were dried and stored in cool conditions for further use. The plant materials and their powders were shown in the fig 1. and 2.

Phytochemical Screening

Qualitative Analysis of Bioactive Compounds

**Author for Correspondence: pudayasri@gmail.com*

Figure 1: *Senna alexandrina* leaf

figure 2: *Senna alexandrina* leaf powder

Table 1: Results for the preliminary phytochemical analysis of selected medicinal plant.

S. No	Phytochemicals	SA AE	SA ME	SA AtE	SA EAE
1.	Alkaloids	+	+ +	+	+
2.	Carbohydrates	+	+ +	+	+
3.	Glycocides	+	++	+	--
4.	Saponins	+	+ +	--	--
5.	Steroids	+	+ +	+	+
6.	Phenols	++	+ +	--	+
7.	Tannins	++	+ +	--	--
8.	Flavonoids	++	+ +	+	++
9.	Protein& Amino acids	+	+ +	+	+
10	Diterpenes	+	+	--	+

SA- Senna alexandrina: AE-Aqueous extract, ME-Methanolic extract, AtE- Aetone extract, EAE- Ethyl acetate extract. + less ++ moderate + ++strong

Table 2: Results of Total Alkaloids Estimation of Selected Medicinal Plant.

S. No	Name of Phytochemical compound	*Senna alexandrina* Methanol extract Absorbance(nm)	Amount*
1	Alkaloids	0.457	0.483

Amount is given in as mg of the phytochemical compound present in one gram of the plant extract.

Phytochemical analysis was carried out for *S.alexandrina* extracts as per the standard methods. Detection of Alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids. Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids. Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube formation of the violet ring at the junction indicates the presence of Carbohydrates. Benedict's test: Filtrates were treated with Benedict's reagent and heated gently orange red precipitate indicates the presence of reducing sugars. Fehling's Test: Filtrates were hydrolyzed with dil. HCl and then the extract is neutralized with alkali and it is heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of Glycosides: Extracts were hydrolyzed with dil. HCl, and then tests were performed for the detection of glycosides. Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of Benzene. The Benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of Saponins by Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of Phytosterols by Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of Triterpenes. Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride and are boiled and cooled which was added with Conc. Sulphuric acid. Formation of brown ring at the junction indicates the presence of phytosterols.

Table 3: Results of Total Flavonoids Estimation in Selected Plant Extract.

S. No	Name of Phytochemical compound	*Senna alexandrina* Methanol extract	
		Absorbance(nm)	Amount*
1	Flavonoids	0.162	0.616

*Amount is given in as mg of the phytochemical compound present in one gram of the plant extract

Table 4: UV-VIS Peak values of *Senna alexandrina* extract.

S.No	*Senna alexandrina*	
	Nanometer	Absorption value
1.	449.01	2.727
2.	532.00	0.837
3.	606.0	0.589
4.	665.0	1.276

Detection of Phenols by Ferric Chloride Test: Extracts were treated with 3-4 drops of Ferric chloride solution. Appearance of bluish black colour indicates the presence of phenols. Detection of Tannins by Gelatin Test: 1% gelatin solution containing sodium chloride was addedto the extract and the formation of white precipitate indicates the presence of tannins. Detection of Flavonoids by Alkaline Reagent Test: Extracts were treated with few drops of Sodium hydroxide solution. Formation of intense yellow cooler, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids. Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and Aminoacids by Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins. Ninhydrin Test: 0.25% w/v Ninhydrin reagent was added to the extract and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Diterpenes by Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of Copper acetate solution. Appearance of emerald green colour indicates the presence of Diterpenes.

Quantitative analysis of selected bioactive compounds

Estimation of alkaloids

Instrumentation

Techcomp UV-2301 UV-Visible Spectrophotometer was employed for the quantitative estimation of phytochemical compounds from the Plant extract, with the help of Hitachi software that attached with the Spectrophotometer. Standard cuvettes were used with the path length of 10mm. Samples were agitated using Ultrasonicator and for weighing the samples, Denver electronic analytical balance (SI-234) was used at various stages of the experiment.

Chemicals and reagents

The chemicals used for the estimation are Sodium phosphate, citric acid, Bromocresol green and Chloroform. All these reagents are of laboratory grade and were purchased from Merck chemicals private limited, Mumbai, Fisher scientific, Mumbai and SD fine chemicals Ltd., Mumbai.

Preparation of reagents

Phosphate buffer solution (pH 4.7)

The buffer solution was prepared by adjusting the pH of 2M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M Citric acid (42.02 g citric acid in 1L distilled water).

Sample Preparation

10 mg of the plant extract was taken and is dissolved in the 10ml of Ethanol which is sonicated for 10minutes. From this 1ml of the extract was taken and was employed for further analysis.

Standard Preparation

Atropine was taken as a standard for the estimation of the Alkaloid content in the plant extract. 10mg of Atropine was taken which is dissolved in 10ml with the suitable solvent i.e., methanol and it is sonicated for 5minutes. This is the stock solution of concentration of 1000 µg/ml which was kept aside for further use. From the stock solution, 1ml was taken and 9ml of same solvent is added to obtain the concentration of 100µg/ml solution and then again 1ml was taken from this which is diluted to 10ml with the same solvent to get 10 µg/ml solutions. This process was repeated till the concentration of 1 µg/ml is obtained.

Preparation of Standard Calibration curve

Calibration curve was prepared by taking 2ml, 4ml, 6ml, 8ml, 10ml and 12ml solutions from the concentration of 10 µg/ml and applying the procedure for the estimation of Alkaloids and the standard curve was plotted by taking the sample concentration on X-axis and absorbance values on Y-axis.

Procedure for the estimation of Alkaloid content

1ml of the extract was added with 5 ml of phosphate buffer (pH 4.7) and 5 ml BCG solution and the mixture was shaken with 4 ml of Chloroform. The extract was collected in a 10-ml volumetric flask and is diluted to makeup the final volume with Chloroform. The blank was prepared as above but without the extract and the absorbance of the complex in chloroform was measured at 470 nm against the blank. Atropine was used as a standard and the results of the assay were compared with Atropine equivalents.

Estimation of Flavonoids

Standard Preparation

Quercetin was taken as a standard for the estimation of the Flavonoid content in the plant extract. 10mg of Quercetin was taken and dissolved in 10ml of the Methanol and it is sonicated for 5minutes. This is the stock solution of 1000 µg/mlconcentration which was kept aside for further use. 1ml of this solution was taken and 9ml of solvent is added to obtain thesolution with a final concentration of 100µg/ml from which 1ml was taken and is further diluted with the solvent to get the solution with 10 µg/ml concentration. The same process was repeated till the concentration of 1 µg/ml was obtained.

Preparation of Standard Calibration curve

Calibration curve was prepared by taking 1ml, 2ml, 3ml, 4ml, 5ml and 6ml solutions from the concentration of 1 µg/ml and applying estimation procedure of Flavonoids. A graph was plotted by taking absorbance on Y-axis and

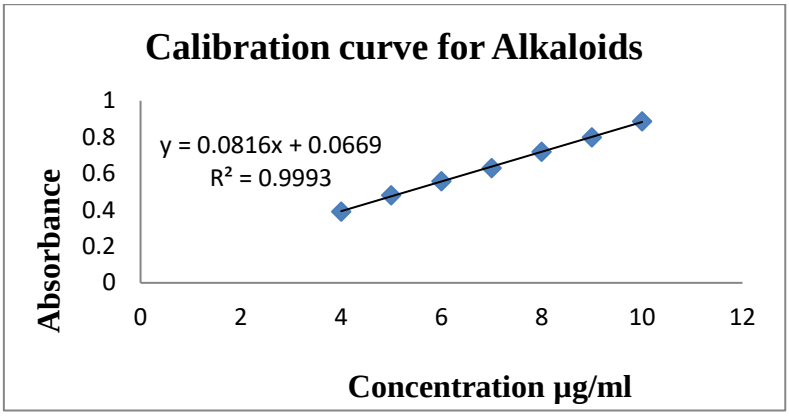

Figure 3: Standard calibration curve for the estimation of Alkaloids in selected plant extract.

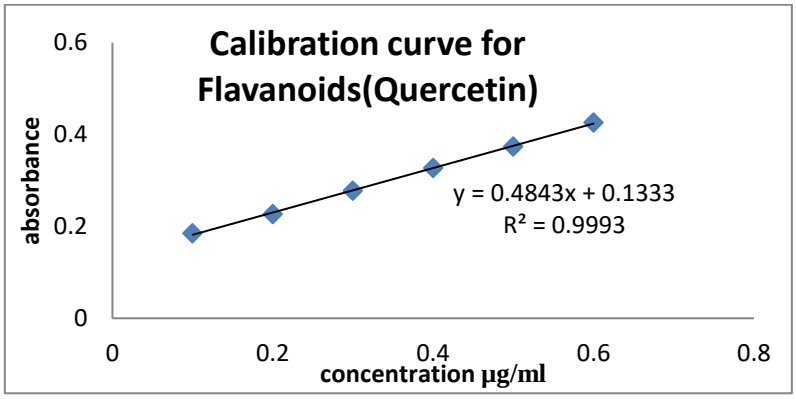

Figure 4: Standard Calibration Curve for the Estimation of Flavonoids in the Selected Plant Extract.

concentration on X-axis. The values obtained are substituted in the regression equation i.e., $Y=mx+c^2$. Here Y is average absorbance of the unknown sample, term m shows slope and c indicates intercept.

Procedure for the estimation of total Flavonoid

Total flavonoid content was estimated using Aluminium chloride ($AlCl_3$) according to a known method, using Quercetin as a standard. 1ml of the plant extract was taken in a test tube which is added with 2ml of 5% $NaNO_2$and 3 ml of $AlCl_3$ (10%) was added to this after 5 min. then the reaction mixture was treated with 2 ml of 1 M NaOH another 5 min and finally the reaction mixture was made up to 10 ml with water and the absorbance was measured at 510 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was taken. For the blank, the extracts were replaced with an equal volume of distilled water. The Flavonoids content in extracts was expressed in terms of Quercetin equivalents.

Identification of Bioactive Compounds

UV-VIS Spectroscopic analysis

The extract was examined under UV-visible light for proximate analysis. These spectral measurements are important for screening the crude plant extract for the presence of particular classes of compounds in their identification. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatmann No.1filter paper by using high pressure vacuum pump and were subjected to UV-VIS spectrophotometric analysis. The sample was diluted to 10 times with the same solvent and the extract was analysed in the UV wavelength ranging from 200-800 nm by employing Perkin Elmer Spectrophotometer and the characteristic peaks were detected.

Results of Qualitative analysis of bioactive compounds

The results of the preliminary Phytochemical studies basing on biochemical protocols have confirmed that the extract of the plant showed positive results for more number of Phytochemical components as it was shown in Table 1 and it can be concluded that both the plant contain Alkaloids and Flavonoids predominantly.

Results of Quantitative analysis of the selected Phytochemical compounds from Senna alexandrina plant extract

The quantitative analysis of the same plant extract by UV Visible spectrophotometric method with Bromocresol green and Atropineas the standard for alkaloids and Aluminium chloride method with quercetin as standard for Flavonoids were carried out. The standard calibration curves obtained for the estimation of Alkaloids and Flavonoids were given in the Figure 3.5 and 3.6 respectively.

Basing on the results of quantitative analysis it was confirmed that leaf extract of *Senna alexandrina* contains 0.483mg/g of alkaloids and0.616mg/g of Flavonoids respectively as it was shown in the Tables 3.2 and 3. 3.

Results of the Bioactive compound identification by UV-Visible Spectrophotometric Analysis

The UV-VIS profile of plant extract was analysed at 200 to800 nm wavelength to identify the compounds containing σ- bonds, π-bonds, andlone pair of electrons, chromophores and aromatic rings. The profile showed the

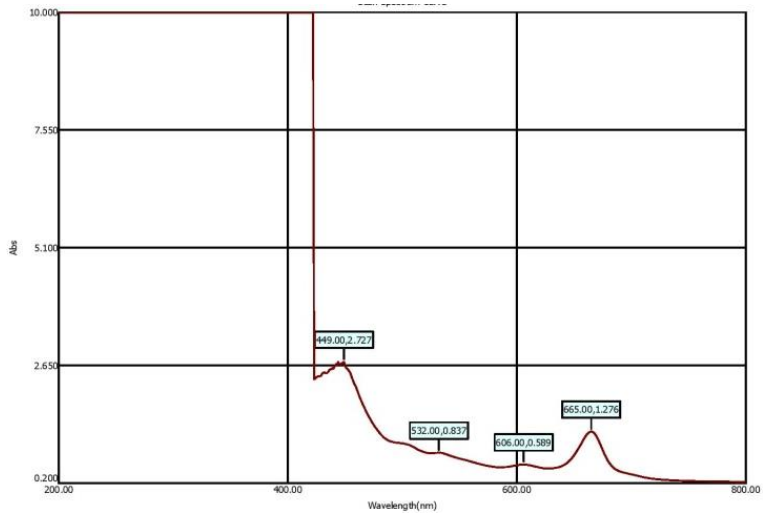

Figure 5: UV-Visible spectrum of *Senna alexandrina* leaf methanolic extract.

peaks at 449.01nm, 532.00and 606.0, 665.0 nm with the absorbance values of 2.727, 0.837, 0.589 and 1.276 *Senna alexandrina* Table 4 Occurrence of peaks at 234-800 nm reveals the presence of phenolic, flavonoids and alkaloids in the leaf extract of *Senna alexandrina*.

In the present study, all the four extracts of the selected plant were preliminarily screened for the phytochemicals and it was found that among them, the methanolic extract was rich in all the phytoconstituents followed by ethyl acetate extract and the selected medicinal plant species, *Senna alexandrina* were further explored for their different bioactive efficacies. The results of the preliminary phytochemical studies, basing on biochemical protocols have confirmed that the extracts of the plants how positive results for more number of phytochemical components as shown in Table 3.1 and it can be concluded that both the plant contain Alkaloids and Flavonoids predominantly in methanolic and ethyl acetate extracts. These phytochemicals could contribute to the various medicinal applications of *Senna alexandrina* plant. The current results are in line with[7] that the secondary metabolites (phytochemicals) and other chemical constituents of the medicinal plant are responsible for their medicinal values. In a previous report on the phytochemical screening of methanolic extract of root and stem of *Eucalyptus globules* showed the presence of alkaloids, flavonoids, saponin, tannins and phenols which was also subjected for the evaluation of antioxidant activity[8]. Similar work was carried out by Abiodun Humphrey Adebayo[9] which is related to phytochemical screening and the determination of antioxidant property in *ChrysophyllumAlbidum* (L). Basing on the results of quantitative analysis it was confirmed that the fruit extract the leaf extract of *Senna alexandrina* contains 0.483 mg/g of alkaloids and0.616 mg/g of Flavonoids respectively. These extracts are involved in scavenging free radicals from tissues, thus reducing the oxidative stress. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Similarly, terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants[10]. Many researchers have reported the presence of certain phytochemicals as responsible for the treatment of specific diseases. Tannins and flavonoids are known to be present in the extracts used as antibacterial and antioxidant agents[11]. Flavonoids and glycosides are also known to prevent cardio-vascular diseases and ulcers[12]. The presence of alkaloids in plant extracts are also used for wide range of pharmacological activities including antimalarial, antiasthma, anticancer, etc.[13] Recent studies also showed that tannins containing extract was used to treat haemorrhoids[14], as antiviral[15] and antiparasite[16 ,17]. also indicated that the presence of these secondary metabolites in plant which produces some biological activities responsible for their potential use as drugs.

The qualitative UV-VIS profile of methanolic extract of both the plant was analysed with in the wave length ranging from 200 to 800 nm. The profile has shown the peaks at 449.01nm, 532.00 and 606.0, 665.0 nm with the absorbance values of 2.727, 0.837, 0.589 and 1.276 respectively for *Senna alexandrina* and Table3. 4. Fig-3.7 & 3.8 Thus the phytochemical profile showed the occurrence of peaks with in the 234-800 nm range of UV, which confirms the presence of phenolics, flavonoids and alkaloids in the leaf extract of *Senna alexandrina*[18, 19,20,21,22].

REFERENCES

1. Chadha, K.L., (2011): Hand Book of Horticulture, Senna, ICAR, New Delhi, pp 622.
2. Kittisupamongkol, W., Nilaratanakul, V., Kulwichit, W., (2008): Near-fatal bleeding, senna, anthe opposite of lettuce. Lancet, 371:773-784.
3. T. H. El-Morsy. (2013): "Antibiotic Properties of Leaf Extracts of Sennaalexandrina (L)". J Am Sci9(1):288-292]. (ISSN: 1545-1003.
4. Borrelli, F. and Izzo, AA. (2000): The Plant Kingdom as a Source of Anti-ulcer Remedies. Phytotherapy Research, 14(8):581–591.
5. James A. Duke et al., (2008): Duke's Handbook of Medicinal Plants of the Bible). CRC Press is an imprint of the Taylor & Francis Group, Boca Raton London New York. 424-428.

6. D. Balasankar et al., Senna – (2013): A Medical Miracle Plant, Journal of Medicinal Plants Studies 1 (3): 41- 47.
7. Varadarajan, P., Rathinaswamy, G. and Asirvatahm, D. (2008): "Antimicrobial properties and phytochemical constituents of rheo discolor. *Ethnobotanical Leaflet,"*12:841-845
8. Zakia Bey-Ould Si Said, Sakina Slimani, HocineRemini, Hayat Idir-Himed, Jean-Paul Mazauric, KhodirMadani, Lila Boulekbache-Makhlouf "Phytochemical analysis and antioxidant activity of *Eucalyptus globulus'*: A comparative study between fruits and leaves extracts SDRP Journal of Chemical Engineering & Bioanalytical Chemistry 1(1), 1-10.
9. Abiodun Humphrey Adebayo, Amos OlalekanAbolaji, Roseline Kela, OlayemiOluseunAyepola, TitilayoBanwoOlorunfemi and Olugbenga Samson Taiwo (2011): "Antioxidant Activities of the Leaves of *ChrysophyllumAlbidum*", G. Pak. J. Pharm. Sci.,24(4):545-551.
10. Soetan, K.O. (2008): "Pharmacological and other benefits of antinutritional factors in plants". A. Review, *African Journal of Biotechnology*, **7**:4713-4721.
11. Cook MC (1996): Flavonoids-Chemistry, metabolism, cardio, protective effects and dietary sources. Nutr. Biochem. 7:66-76.
12. Swigio G, Tyrakwoska B (2003): "Quality of commercial apple juices evaluated on the basis of polyphenol content and TEAC antioxidant activity". J. Food Sci. 68(5):1844-1849.
13. Kittakoop P, Mihidol C, Ruchirawat S (2014): Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis and smoking cessation. Curr. Top Med. Chem. 14(2):239-255.
14. Njoku PC, Akumufula MI (2007): Phytoche) mical and nutrient evaluation of Spondiiasmombi Leaves. Pak. J. Nutr. 6(6):613-615.
15. Lu L, Liu SW, Jiang SB, Wu SG (2004): Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol. Sin. 25(2):213-8.
16. Kolodziej H, Kiderlen AF (2005): Antileishmanial activity and immune modulatory effect of tannins and related compounds on leishmania parasitized RAW 264.7 cells. Phytochemistry 66(17):2056-2071.
17. Sofowora A (1986): The state of medicinal plants research in Nigeria. University Press, Ibadan, Nigeria.
18. Jasper C, Maruzzella JC, Henry PA. (1958): The antimicrobial activity of perfume oils. J Am Pharm Asso 47:471-476.
19. Sofowora A. (1993): Medicinal Plants and Traditional Medicine in Afric. John Wiley and son Ltd 150-153.
20. Amir Muhammad Khan, Rizwana Aleem Qureshi, Faizan Ullah, Syed AneelGilani,AsiaNosheen, SumairaSahreen, Muhammad Khan Laghari, Muhammad Yousif Laghari, Shafiq-Ur-Rehman1, Ishtiaq Hussain and Waheed Murad.(2011):"Phytochemical analysis of selected medicinal plants of *Margalla Hills* and surroundings Journal of Medicinal Plants Research" 5(25):6017-6023.
21. Thite SV., Chavan YR., Aparadh VT and Kore BA. (2013): "Preliminary Phytochemical Screening of Some Medicinal Plants". IJPCBS 3(1):87-90.
22. Minakshi B, Jharna D, Chanbi Devi E, Nayan T, Partha PK, Kundal N and Manash(2016): Phytochemical Analysis of Traditional Medicinal Plants and their Antimicrobial Activity: An Experience from North East India Open Access Journal of Pharmaceutical Research 1(1):1-7.