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Research Paper

Quantitative Phytochemical Estimation and Antioxidant Potentials of *Borreriascabra*

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ABSTRACT
The quantitative phytochemical estimation of the secondary metabolites and the antioxidant potentials ofthe whole plant of Borreriascabra were evaluated in this study. The quantitative estimation of the bioactive molecules using standard methods showed the presence of saponin (15.15%), terpenoids (5.51%), flavonoids (4.47%), alkaloids (6.08%), tannins (0.32%) and phenols (0.29%). The quantities of saponins, terpenoids, flavonoids and alkaloids are presentin moderate amounts while tannins and phenols are present in traces. The presenceof phytochemicals can contribute positively to the medicinal values of the plant. The radical scavenging activities of the plant extracts against 2, 2 – Diphenyl -1- picrylhydrazyl radical (DPPH) were determined by UV spectrophotometer at 517 nm. The highest percentage inhibition of 54.97% was recorded at a concentration of 0.05mg/ml as compared to the 93.13% exhibited by vitamin C at the same concentration. The lowest antioxidant activity of the plant extract was shown at a percentage inhibition of 20.36% having a concentration of 6mg/ml as compared to the lowest percentage inhibition of vitamin C (84.20%) which reveals that the plant extracts have lower scavenging ability than vitamin C. The results may suggest that the plant can serve as a good source of natural antioxidants.Studies on the isolation and characterization of the bioactive compounds from the plant are in progress.

KEY WORDS: Quantitative phytochemicals, medicinal plants, antioxidant and Borreriascabra.

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I. INTRODUCTION

The use of plant to meet health-care needs has greatly increased worldwide in the recent times. Plants that possess therapeutic properties and exerting beneficial pharmacological effects on the animal body are generally designated as medicinal plants (Vishnu *et al.*, 2011). In actual fact, plants were the mere basis of healing till the initiation of manufactured pharmaceutical products during previous years (Salem, 2017). The use of natural bioactive molecules by humans has an advantage in that they have a milder side effect on the body as compared to chemically synthesized drugs **(**Badisa*et al*.,2003).

The investigation of plants for bioactive secondary metabolites has become inevitable due to significant correlation between their uses in traditional medicine and the observed biological effects of their extracts (Priyanka and Daljit, 2017).

There has been an increased interest globally to identify natural antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry(Priyanka and Daljit, 2017)**.**Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases(Krishnaiah*et al*, 2011).

BorreriaScabra is a dicotyledon belonging to the Rubiaceae family and the genus is *Borerria*. The order is GentianalesJuss. and superorder Asteranae with class Equisetopsida. *Borreriascabra* is called Damfarkami by the Hausa people of Nigeria and has been used extensively in the treatment of malaria in the northern part of Nigeria. Rubiaceae family is a large family of 630 genera and about 13000 species found worldwide, especially in tropical and warm regions. These plants are not only ornamental but they are also used in African folk medicine to treat several diseases. Indeed, more than 60 species are used for more than 70

medicinal indications including malaria, hepatitis, eczema, oedema, cough, hypertension, diabetes and sexual weakness (Simplice*et al.*, 2011).

The qualitative phytochemical constituents and the *in-vivo*antiplasmodial activity of *Borreriascabra* has been reported by Apampa*et al.*, (2019). The need to explore the antioxidant potential of the plant vis-à-vis the quantification of the phytochemicals has necessitated this study.

II. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant

The whole plant of *Borreriascabra* was collected from Ahmadu Bello University Farms, Zaria in Kaduna State, Nigeria in October 2015. The plant was authenticated by MallamNamadiSanusi (a taxonomist with Ahmadu Bello University) and the voucher specimen of the plant samples were deposited at the herbarium of the Department of Biology, Ahmadu Bello University, Zaria, with voucher number 2811.

2.2 Extraction of Plant Sample

The whole plant of *Borreriascabra* was air dried and ground using a clean pestle and mortar. Fine powder of the plant samples weighing 250g each were percolated in 1.2 litres of 96% ethanol each in separate bottles for two weeks. The percolates were decanted and concentrated using rotary evaporator at 40 °C. The extracts were stored in a refrigerator at 4 °C in well-closed containers.

2.3 Quantitative Determination of PhytochemicalConstituentsof*Borerriascabra*

In the quantitative determination of all the phytochemicals, the percentage of the estimated phytochemical is calculated using:

$$\% \text{ Phytochemical} = \frac{\text{Weight of phytochemical}}{\text{Weight of sample}} \times 100$$

2.3.1 Determination of Alkaloids: Quantitative determinationof alkaloid was carried out according to the protocolsdescribed by Harborne (1973). Exactly 200 cm^3 of 10% acetic acid in ethanol was added to each powdered sample (2.00 g) in a 250 cm^3beaker, covered and allowed to stand for 4 hours. This was filtered and the extract wasconcentrated on a water bath to one-quarter of the originalvolume followed by addition of 15 drops of concentratedammonium hydroxide dropwiseto the sample until theprecipitation was completed. After3 hours of mixture sedimentation, the supernatant was discardedand the precipitates were washed with 20 cm^3 of 0.1Mof ammonium hydroxide and then filtered using Whatmanfilter paper. The residue is the alkaloid which was dried in an oven and weighed.

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100$$

2.3.2 Determination of Saponin: The plant sample (20g)was dispersed in 500 ml of 20 % ethanol. The suspension washeated over a hot water bath for 4 hours with continuous stirringat about 55 °C. The mixture was filtered and the residue re-extractedwith another 200 ml of 20% aqueous ethanol. The combinedextracts were reduced to 40 ml over water bath at about 90 °C.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 layerwas discarded. The purification process was repeated. 60 ml of n-butanolwas added and the combined n-butanol and the extracts were washed twice with10 ml of 5 % aqueous sodium chloride. The remaining solutionwas heated in a water bath. After evaporation, the samplewas dried in the oven into a constant weight. The saponin content was calculated as a percentage as reported by Obdoni and Ochuko (2001).

2.3.3 Determination of Flavonoids. The flavonoid content of the plant samples was determined by the method reported by Ejikeme et al., (2014) and Bohmand Koupai(1994). Exactly 50 ml of 80% aqueous methanol was added to 2.00 g of sample in a 250 ml beaker, covered,and allowed to stand for 24 hours at room temperature.After discarding the supernatant, the residue was re-extracted(three times) with the same volume of ethanol. Whatman filter paper was used to filter whole solution of each sample. Each sample filtrate was later transferred into a crucible and evaporated to drynessover a water bath. The content in the crucible was cooled ina desiccator and weighed until constant weight was obtained.
The percentage of flavonoid was thus calculated.

2.3.4 Determination of Terpenoids: About 2.00g of the plant sample was weighed and soaked in 50 ml of 95% ethanol for 24 hours. The extract was filtered and the filterate was extracted with petroleum ether and concentrated to dryness. The dried ether extract was treated as percentage of total terpenoids(Ferguson,1956).

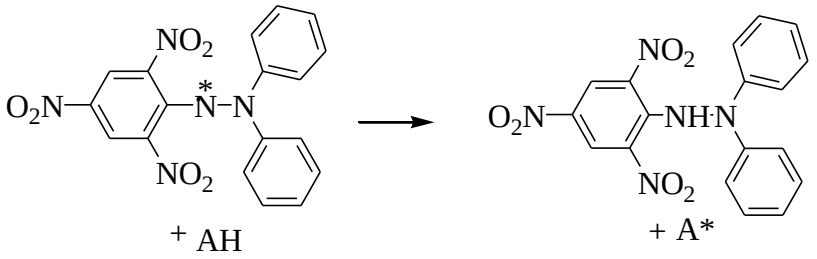
2.3.5 Determination of Phenolic Compounds: The extract(500mg) was dissolved in 100 ml of triple distilled water (TDW). The solution (1 ml) was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with

TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (Hagerman et al., 2000).

2.3.6 Determination of Tannins: The sample (500 mg) was weighed into a 50 ml capacity plastic bottle. Exactly50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filterate was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.IM HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mins.The values obtained were plotted against various diluted concentrations of gallic acid. (Van-Buren and Robinson, 1981).

2.4 Determination of Antioxidant Activity

The radical scavenging activities of the plant extracts against 2,2 Diphenyl-1-picryl hydrazyl Radical, DPPH (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm (scheme 1).



2,2 Diphenyl-1-picryl hydrazyl Radical (DPPH) Deep Violet **Diphenylpicryl hydrazine Yellow**

Scheme 1: Scavenging reaction of DPPH.

This method depends on the reduction of purple DPPH to a yellow coloureddiphenylpicrylhydrazine and the remaining DPPH which showed maximum absorption at 517nm was measured. Exactly I ml of the extract was placed in a test tube, and 3ml of methanol was added followed by 0.5 ml of 1mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH without the sample. Vitamins C were used as the antioxidant standard at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/ml. The experiment was performed in triplicates. The higher the scavenging ability value, the higher the antioxidant activity detected in the methanol extracts (Shimada et al., 1992). The radical scavenging activity was calculated using the formula below:

% inhibition = {[Ab-Aa]/Ab} x 100 ……(1)

WhereAb is the absorption of the blank sample and Aa is the absorption of the extract. Percentage of radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC_{50} value, which is defined as the amount of antioxidant substance required to scavenge 50 per cent of free radicals present in the assay system. IC_{50} values are inversely proportional to the antioxidant potential. A smaller IC_{50} value means higher antioxidant value.

III. RESULTS AND DISCUSSION

3.1 Quantitative Estimation of Phytochemicals in *Borreriascabra*

Quantitative determination of the phytoconstituentsof *B.scabra* extracts was carried out for the powdered plant materials by various standard methods. The knowledge of the chemical constituents of plants is desirable because such information is valuable in the synthesis of complex chemical substances (Parekh and Chanda, 2007). From the results (table 1, figure 1), the saponin content (15.15%) was found to be the highest in the plant followed by alkaloids(6.08%), terpenoids (5.51%) and flavonoids (4.47%). The lowest values of 0.32% and 0.29% were recorded in tannins and phenols respectively. The results revealed that some of the secondary metabolites are present in trace amounts but does not connotes or signify their total absence despite the fact that their presence may not be detected by qualitative analysis. The phytochemicals present in plants are responsible for preventing diseases and promoting health care due to their underlying mechanism of action. Researchers have identified and isolated the chemical components and established biological potency of these secondary metabolites through *in vitro* and *in vivo* studies in experimental animals and through epidemiological and clinical-case control studies in man (Saxena*et al.*, 2013). Phytochemicals may reduce the risk of coronary heart disease by preventing the synthesis or absorption of cholesterol. They normalize blood pressure and clotting, improve arterial elasticity and can detoxify substances that cause cancer(Akunyili, 2003). They appear to

neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. They have also been promoted for the prevention and treatment of malaria, diabetes, high blood pressureetc, (Saxena*et al.*, 2013). Their classification by function shows that an individual compound may have more than one biological function serving as both an antioxidant and antibacterial agent or more. This study tends to correlate relationship of these secondary metabolites to possible biological activities and evaluate it as a potential source of natural bioactive chemicals.

Table 1: Results of the Quantitative Phytochemical analysis of *Borreriascabra*

Plants	Saponin (%)	Terpenoid (%)	Flavonoid (%)	Alkaloid (%)	Tannin (%)	Phenol (%)
Borreriascabra	15.15	5.51	4.47	6.08	0.32	0.29

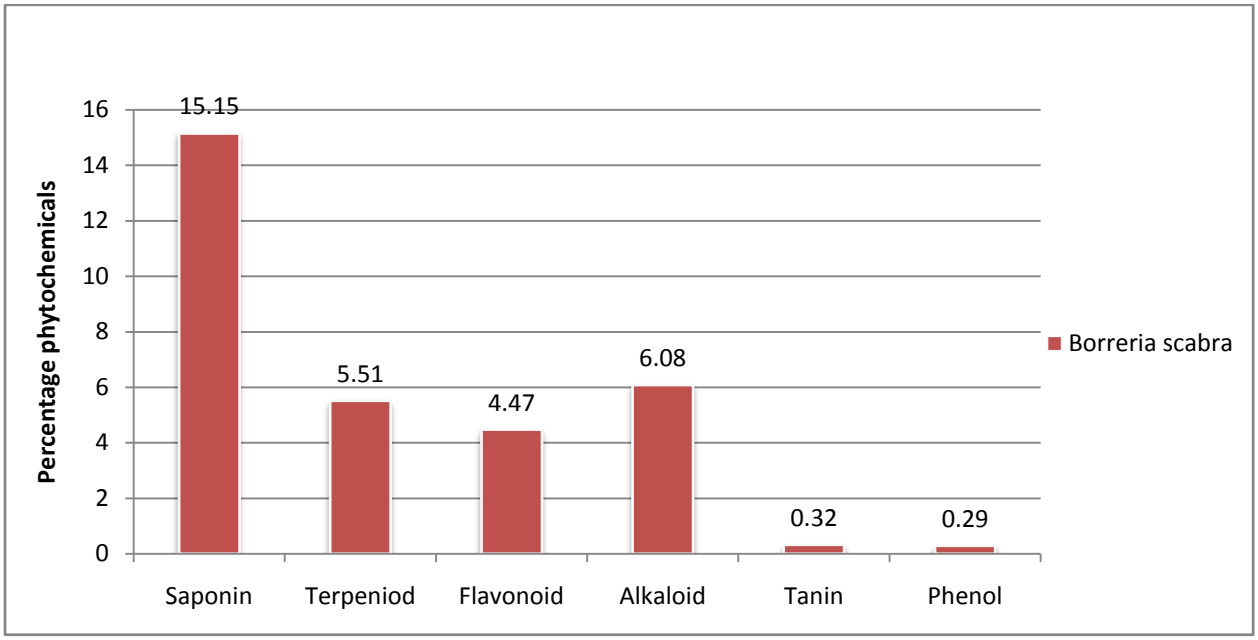


Fig 1: Percentage estimation of phytochemicals in *Borreriascabra*

The DPPH test provides information on the reactivity of the extract with a stable free radical which gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow (scheme 1). The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract (Ayoola*et al.*, 2008). Anti-oxidant effects in the ethanol extract of *Borreriascabra* are dose related and decreases as concentration increases, Table 2 shows a noticeable variability in the antioxidant activity of the plant extract, as highest percentage inhibition of 54.97% was recorded at a concentration of 0.05mg/ml as compared to the 93.13% which was exhibited by Vitamin C at same concentration. The lowest antioxidant activity of the plant extract was shown at a concentration of6mg/ml witha percentage inhibition of 20.36% as compared to the lowest percentage inhibition of Vitamin C of 84.20% at same concentration. The results revealed that the plant extract activity when compared to Vitamin C is lower than and not as potent in scavenging ability as Vitamin C. This results agrees with whatwas reported by Olutayo*et al.*, (2011) which shows that the root, wood and stem of *E.angolense*has an antioxidant activity which are dose related and decreases as concentration increases, although IC_{50}value above 1 mg/ml of the extract seems to possess higher antioxidant capacity at lower concentration (81.34 % at 0.05 mg/ml).

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. They are of great importance to the health of individuals and communities. Thus, it is clear that antioxidants in plants play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phytoconstituents in them (Anjali and Sheetal, 2013).

Table 2: Table of the Results of Antioxidant Activity of Ethanol Extract of *Borreriascabra*

Concentration (mg/ml)	Absorbance(nm)	% Inhibition	% Inhibition of Vitamin C

	1	2	3	Average		
6	0.352	0.351	0.353	0.352	20.36	84.20
5	0.348	0.350	0.349	0.349	21.04	85.00
4	0.276	0.275	0.274	0.275	37.78	89.51
3	0.269	0.271	0.270	0.270	38.91	90.01
2	0.251	0.253	0.252	0.252	42.98	90.41
1	0.241	0.243	0.242	0.242	45.24	90.19
0.5	0.221	0.220	0.220	0.220	50.22	91.33
0.05	0.201	0.198	0.199	0.199	54.97	93.13

Absorbance of Blank at 517nm is **0.442nm.**

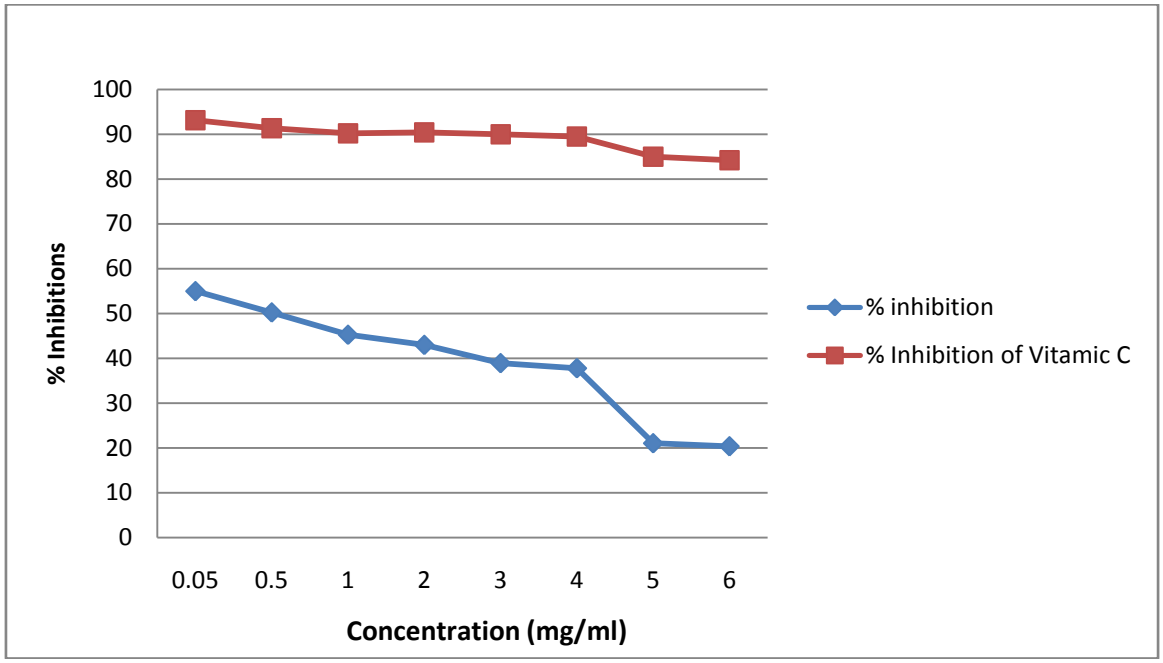


Fig 2 : Antioxidant Activity of Extracts of *Borreriascabra*

IV. CONCLUSION

In the present study, quantitative phytochemical estimation of *Borreriascabra* reveals the presence of saponins, terpenoids, flavonoids and alkaloids in moderate amounts and tannins and phenols were found in traces. The presence of the phytochemicals has affirmed the use of *B.scabra* in treating malaria and other ailments. The plant could also be a potential source of natural antioxidant with therapeutic properties that could prevent or slow down the ageing process associated with oxidative stress and related degenerative diseases in humans. Further studies to isolate, identify andelucidate the structures of the bioactive compounds in *B. scabra* are in progress.

REFERENCES

[1]. Akunyili, D.N. (2003).The Role of Regulation of Medicinal Plants and Phytomedicine in Socio-Economic Development, AGM/SC of the Nigerian Society ofPharmacognosy. Abuja, Nigeria. 1-7.

[2]. Anjali, S, and Sheetal, S. (2013).Phytochemical Analysis and Free RadicalScavenging Potential of Herbal and MedicinalPlant Extracts.*Journal of Pharmacognosy and Phytochemistry.* 2 (4): 22-29.

[3]. Apampa, S.A,Adoum, O.A and Ameh, F.S. (2019).Antimalarial Activity of Alysicarpuszeyheri(Harv), Borreriascabra and Uapacapilosa (Hutch) Extracts on Mice Infected with *Plasmodium Berghei*Berghei.*European Journal of Biomedical and Pharmaceutical sciences*. 6(1): 607-614

[4]. Ayoola, G.A., Coker, H.A., Adesegun S.A., Adepoju-Bello,A.A., Obaweya, K, Ezennia, E.C and Atangbayila, T.O. (2008). Phytochemical Screening and Antioxidant Activities of some selected Medicinal Plants used for Malaria Therapy in South western Nigeria. *Trop.J. Pharm. Res*., 7: 1019-1024.

[5]. Badisa, R.B, Tzakou, D, Couladis, M, Pilarinou, E. (2003).Phytotherapy Research "Cytotoxic Activities of some Greek Labiatae Herbs". 17(5). Wiley & Sons Ltd, U.S.A. 472-475.

[6]. Bohm, B.A and Koupai–Abyazani, M.R. (1994).Flavonoids and Condensed Tannins from Leavesof Hawaiian *Vacciniumreticulatum* and *V.calycinum* (Ericaceae).*Pacific science*.48(4): 458 – 463

[7]. Ejikeme, C.M. Ezeonu, C.S and Eboatu, A.N (2014).Determination of Physical and Phytochemical Constituents of some Tropical Timbers indigenous to Niger Delta Area of Nigeria, *European Scientific Journal*. 10 (18) :247–270.

[8]. Ferguson, N. M. (1956). A Text book of Pharmacognosy. Mac Milan Company, New Delhi. India. 191.

[9]. Hagerman, A., Muller, I and Makkar, H. (2000).Quantification of Tannins in Tree Foliage. A Laboratory Manual, Vienna: FAO/IAEA.Pp 4-7

[10]. Harborne, J.B (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, UK. Pp. 49-188.

[11]. Krishnaiah D, Rosalam S, Nithyanandam, R (2011). A Review of the Antioxidant Potential of Medicinal Plant Species.*Food and Bioproducts Processing*. 89. 217–233.
[12]. Obdoni, B.O. and Ochuko, P.O. (2001). Phytochemical Studies and Comparative Efficacy of the Crude Extracts of some Homostatic Plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci*. 8: 203-208.
[13]. Olutayo, O,Doyinsola, I, Simon, O, Abayomi, O and Thomas, S.C. (2011).Phytochemical and Antioxidant Properties of some Nigerian Medicinal Plants.*AmJ. Sci.Ind. Res*., 4(3):328-332.
[14]. Parekh, J, and Chanda,S.(2007). Antibacterial and Phytochemical Studies on Twelve Species of Indian Medicinal Plant .*African Journal of Biomedical Research*. 10(2): 175 – 181.
[15]. Priyanka, C, and Daljit, S.A. (2017). Antioxidant Compounds Derived from Plants, Description and Mechanism of Phytochemical. *Journal of Agroecology and Natural Resource Management.*4(1): 55-59.
[16]. Salem, M.E, Khalifa, A.A, Hanan,S.A,Wesam, A.K, Fouzy, A. (2017).Qualitative and Quantitative phytochemical Analysis and Antimicrobial Activity of "Retama" Extract Grown in ZlitenLibya.*International Journal of Medical Science and Clinical Inventions*. 4(4):2861-2866.
[17]. Saxena, M, Saxena, J, Nema R, Singh, D, and Gupta, A.(2013).Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry.*1(6): 168-182.
[18]. Shimada, K, Fujikawa, K, Yahara, K, and Nakamura, T. (1992).Antioxidative Properties of Xanthan on the Autoxidation of Soyabean Oil in Cyclodextrin Emulsion. *J. Agric. Food Chem.* 40: 945-948.
[19]. Simplice, D. Karou, TchadjoboTchacondo, Denise, P. Ilboudo, and Jacques, S. (2011). Sub Saharan Rubiaceae: A Review of Their Traditional Uses. Phytochemistry and Biological Activities.*Pakistan Journal of Biological Sciences*. 14 (3): 149-169.
[20]. Van-Buren, T. and Robinson, W. (1981).Formation of complexes between Protein and Tannin acid.*J. Agric. Food Chem*. 17(4): 772-777.
[21]. Vishnu, P.P,Radhika, R, Siva, K, Ramchandra, M, Prameela, D.Y, Srinivas, R.A. (2011).Evaluation of Anti-Cancer Activity of *Tridaxprocumbens* Flower extracts on Pc 3 Cell Lines. *International Journal of Advances in Pharmaceutical Sciences*. 2 (1) : 28-30.