

Research Article

## Phytochemical Profile and Antioxidant Activities of Solvent-solvent Fractions of *Haematostaphis barteri* Hook F. (Anacardiaceae) Stem Bark Extracts

Ezekiel J S\*, Adamu H M, Chindo, I Y, Garba, I H

*Department of Chemistry, Faculty of Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria.*

*Available Online: 12<sup>th</sup> December, 2015*

### ABSTRACT

*Haematostaphis barteri* is a plant used in the northern part of Nigeria to manage degenerative diseases such as cancer, anemia and hemorrhage. The dried powdered stem bark was sequentially extracted with soxhlet using solvents of varying polarities. Phytochemical screening of the extracts using standard methods revealed the presence of flavonoids, cardiac glycosides and tannins as the major phytochemicals detected. The scavenging activities of the various crude extracts on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical was determined and the result showed very low activities with only aqueous methanol, ethanol and acetone extracts showing inhibitions of 48.2%, 51.3% and 49.9% respectively. But when all of the crude extracts were solvent/solvent fractionated, antioxidant activities of the polar and semi-polar fractions improved appreciably. Presence of polyphenolic compounds such as flavonoids and tannins could be responsible for the antioxidant activity of *H. barteri*.

**Keywords:** antioxidant, metabolites, purification, free radicals, anticancer

### INTRODUCTION

There has been a remarkable resurgence of interest in natural products research over the past decade or so. Secondary metabolites have an extensive history of use as therapeutic metabolites occurring as the following compounds: alkaloids, Anthraquinones, coumarins, essential oils (lower terpenoids and phenylpropanoids), flavonoids, steroids and Terpenoids (cardenolides, diterpenes, iridoids, monoterpenoids, sesquiterpenoids (including sesquiterpene lactones) and triterpenoids<sup>1,2</sup>

In this research, the stem bark of *Haematostaphis barteri* Hook. f (Anacardiaceae) was extracted and screened for its phytochemical profiles and for its antioxidant activity. Stem bark of *H. barteri* have been used by traditional healers in northern Nigeria for the management of ailments such as, cancer<sup>3</sup>, stomach ache, and vomiting<sup>4</sup>, anemia and hemorrhoid (Personal interview). The use of this plant in traditional medical practices was corroborated by several published works. Different ethnic groups in Ghana also use the leaves and bark infusion of *H. barteri* in treatment of malaria, hepatitis and sleeping sickness<sup>5</sup>. Similarly<sup>6</sup> reported the use of stem bark extract of the same plant in management of trypanosomiasis in northern Nigeria. The current research attempted to isolate the bioactive compounds responsible for the aforementioned activities.

### MATERIALS AND METHOD

#### *Sample Collection, Identification and Preparation*

Identifiable parts of *Haematostaphis barteri* Hook f. (Anacardiaceae) was collected in January, 2015 from

North Eastern Nigeria, and conveyed to North East Arid Zone Development Programme (NEAZDP), Gashua, Yobe State, Nigeria where identification was duly made by a plant taxonomist. The stem bark of the plant was collected and shade dried on a tray in a well-ventilated room for several days to ensure complete dryness and to make it free from microbial fermentation<sup>7</sup>. The dried material was pulverized in a clean mortar and pestle into powder.

#### *Extraction of Plant Material*

The powdered plant product was subjected to sequential exhaustive soxhlet extraction. Sequential extraction involves using solvents of varying degree of polarities and in this very research extraction followed the sequence; n-hexane, dichloromethane, ethyl acetate, acetone, ethanol and aqueous methanol (1/1 v/v). 100 g of sample was extracted with soxhlet for 6 hours each which yielded six different extract fractions (HX, DCM, EA, AC, EtOH and MeOHaq).

#### *Solvent Fractionation*

The recovered crude extracts from the soxhlet extraction were subjected to purification by a sequential solvent/solvent fractionation with a little modification. Each of the selected crude extracts (EA, AC, EtOH, and MeOHaq) obtained from the previous sequential soxhlet extraction was subjected to sequential solvent fractionation. During this process the extracts were suspended in a solvent, stirred for a short while and filtered. This was repeated about 3 times until filtrate was colourless, then the next solvent with higher polarity was used for similar process. The procedure continued until

\*Author for Correspondence

Table 1: Phytochemical screening of *Haematostaphis barteri* stem bark extracts

Phytochemical Reagents	Solvent Extracts				
	DCM	EA	AC	EtOH	MeOH(aq)
Alkaloids: Meyer's test	-	-	-	-	-
Flavonoids: Lead acetate test	-	+	++	+	+
Saponins: -Frothing test	-	-	-	+	+
-emulsion test	-	-	-	-	-
Cardiac Glycosides: Salkowski's test	++	++	++	++	++
Steroids: Leibermann/Burchard's test	-	-	-	-	-
Terpenoids: Leibermann/Burchard's test	+	+			
Tannins: Braymer's test		+	++	+	+
Anthraquinones: Borntrager's test	-	-	-	-	-
Reducing sugar: Fehling's test	-	-	+	+	+
Phlobatannins	-	-	-	-	-

Key: + = present; ++ = strongly present; - = absent; DCM = dichloromethane; EA = ethyl acetate; AC = acetone; EtOH = ethanol; MeOH(aq) = aqueous methanol extract (1:1, v/v)

Table 2: DPPH Free radical scavenging properties of *H. barteri* stem bark crude extracts

Extracts	Absorbance (517 nm)	Inhibition (%)
DCM	1.036	30.0
EA	0.957	35.7
AC	0.745	49.9
EtOH	0.725	51.3
MeOH(aq)	0.770	48.2
Ascorbic acid	0.030	98.0
Blank	1.488	0.00

that particular crude extract solubilized in the last solvent, then the next extract follows suit.

#### Phytochemical Screening

Phytochemical analysis was performed on the crude extracts using the standard methods<sup>8,9,10</sup> for the qualitative screening of phytochemicals of interest. The phytochemicals that were screened for are: Alkaloids, Flavonoids, Saponins, tannins, Cardiac glycosides, Anthraquinones, steroids, terpenoids, and reducing sugar.

#### Test for reducing sugars: Fehling's test

To about 2ml aqueous solution of the extract in a test tube was added 5ml mixture of equal volumes of Fehling's solution I and II and boiled in a water bath for about 2 minutes. The test tube was observed for brick-red precipitate.

Test for Tannins (Braymer's test): Few drops of extract was treated with 10% alcoholic FeCl<sub>3</sub> solution and observed for bluish green colouration for tannins.

#### Test for saponins

Frothing test: 3ml of the aqueous extract was mixed with 10ml distilled water in a test tube. The tube was stoppered and shaken vigorously for 5 minutes; it was allowed to stand for 30 minutes and observed for honeycomb froth.

Emulsion test: 3ml of the aqueous extract was taken in a test tube, stoppered and shaken vigorously for 5 minutes, after which 2-3 drops of olive oil are added and shaken again. Then it was put to rest for 30 minutes and observed for a layer of emulsion in between the aqueous layer and froth.

#### Test for cardiac glycosides

Salkowski's Test: The extract was dissolved in 2ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at interface indicated steroidal ring (that is aglycone portion).

#### Test for terpenoids/steroids

Leibermann/Burchard's Test: To 2ml of chloroform extract, 2ml of acetic anhydride and few drops of concentrated sulphuric acid were added in a test-tube. Blue-green ring between layers indicate steroids. Pink-purple ring indicate terpenoids.

#### Test for flavonoids

##### Lead Acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow coloured precipitate indicates the presence of flavonoids

#### Test for Alkaloids

1.0g of extract was dissolved in 5ml of 10% ammonia solution and extracted with 15ml of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15ml of dilute sulphuric acid. Meyer's reagent test: 2.3 drops of Meyer's reagent was added to a portion of the acidic solution in a test tube and observed for an opalescence or yellow-white precipitate indicative of the presence of alkaloids.

#### Test for Phlobatannins

Aqueous extract was boiled with 1% aqueous Hydrochloric acid. Red precipitate was indicative of phlobatannins

#### Antioxidant

##### 2, 2-Diphenyl-1-picrylhydrazyl Stable Free Radical Scavenging Assay

The capacity of the extract fractions to scavenge the stable free radical; 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to<sup>11,12</sup>. 2ml of 1mg/ml solution of each extract in 95% methanol were mixed with 2ml of 0.1mM methanolic solution of DPPH. This reaction was conducted in triplicate for right precision of experiment. The mixtures were vortexed thoroughly for one minute at room temperature and incubated for 30 minutes in the dark. Finally, absorbance of each of the mixtures was read at 517nm on a JENWAY 6305 spectrophotometer. The same experiment was conducted with a blank constituted of 95% methanol mixed with the DPPH solution and also with a

Table 3: DPPH Free radical scavenging properties of *H. barteri* stem bark solvent fractions

Extract Fraction	Absorbance at 517 nm, (Mean $\pm$ SD, n = 3)	Percentage Inhibition (%)
EA/EA	0.826 $\pm$ 0.009	47.89
EA/AC	0.156 $\pm$ 0.011	90.16
EA/EtOH	0.758 $\pm$ 0.006	52.18
AC/AC	0.194 $\pm$ 0.003	87.76
AC/EtOH	0.250 $\pm$ 0.001	84.23
EtOH/AC	0.259 $\pm$ 0.002	83.66
EtOH/EtOH	0.208 $\pm$ 0.004	86.88
EtOH/MeOH	0.232 $\pm$ 0.005	85.36
MeOH/EtOH	0.278 $\pm$ 0.007	82.46
MeOH/MeOH	0.240 $\pm$ 0.001	84.86
Ascorbic acid	0.131 $\pm$ 0.001	91.74
Blank	1.585 $\pm$ 0.002	00.00

positive control constituted of 1mg/ml of L-(+)-Ascorbic acid solution in 95% methanol. The antioxidant capacity of each extract sample was expressed in terms of percentage inhibition and was calculated as:

$$\% \text{ Inhibition} = \frac{Ab - Ac}{Ac} \times 100$$

Where, Ab = the absorbance of blank,  
Ac = absorbance of extract or the control.

## RESULT

In order to obtain some information on the active components present in the sequential solvent extracts of the stem bark of *Haematosaphis barteri*, different analytical techniques were employed. Firstly, phytochemical screening was performed on each type of solvent extract and result was presented in table 1. Secondly, antioxidant assay using DPPH free radical was performed on all solvent extracts and result was also presented in table 2 and figure 1. Finally, the solvent extracts were purified by solvent-solvent fractionation and the fractions obtained were subjected to further antioxidant assay and the result was presented in table 3 and figure 2.

## DISCUSSION

The *H. barteri* extracts obtained through sequential soxhlet extraction; DCM, EA, AC, EtOH and MeOHaq were screened for the presence of phytochemicals according to standard methods<sup>8, 9, 10</sup> and, the results were presented in Table 1. Flavonoids, cardiac glycosides and tannins were the prominent phytochemicals in the extracts. Alkaloids were not detected in all of the extracts when tested with Meyer's reagent. These findings agree with<sup>3</sup>. However,<sup>5</sup> detected alkaloids only in a mixture of dichloromethane/methanol extract but did not detect alkaloids in aqueous extract of the stem bark extract of *H. barteri*. Many types alkaloids such as strychnine and coniine are poisonous whereas others are used in medicine in controlled concentrations as pain relievers and as antitussive like morphine and codeine more especially the steroidal alkaloids<sup>13, 14</sup>. The lead acetate test revealed the presence of flavonoids in almost all of the extracts except

in DCM extract. Researchers have repeatedly ascribed antioxidant properties of medicinal plant extracts to flavonoids and phenolic compounds.<sup>15, 16, 17</sup> isolated flavonoids that were responsible for antioxidant and cytotoxic properties of some plants' extracts.<sup>16, 18</sup> reported the isolation of anticancer flavonoids through bioassay guided isolation of antioxidant and cytotoxic compounds. Flavonoids have been shown to have a wide range of biological activities including; anti-allergic, antibacterial, anti-inflammatory, antimutagenic, antioxidant, antiproliferative, antithrombotic, antiviral, hepatoprotective and antihypertensive<sup>14, 15, 19</sup>. Perhaps the traditional use of the stem bark extract of *H. barteri* as reported in literature and personal interviews for the management of cancer, anemia, hemorrhage and other related illnesses could be due to the presence of flavonoids and other phenolic compounds.

Frothing and emulsion tests did not detect saponins in both plants except for slight presence in ethanol and aqueous methanol extracts. This is quite similar to<sup>3</sup> who reported entire absence of saponins in *H. barteri* extracts, but<sup>5</sup> showed presence of saponins in aqueous extract but not in the extract of a mixture of dichloromethane/methanol extracts of *H. barteri*. *Holigarna grahamii* and *pistacia atlanta* are medicinal plants and family members with *H. barteri*. Both plants were reported to contain no saponins<sup>20, 21</sup>. Saponins have been reported to exhibit antifungal, anti-inflammatory, fungistatic and molluscicidal activities<sup>22</sup> and some activities of saponins include anti-inflammation, antiparasitic, and antiviral. It has also been shown to kill tumour cells,<sup>23</sup>. Cardiac glycosides were tested for by the Salkowski's test and found to be positive. But both<sup>3, 5</sup> did not detect glycosides in the stem bark extracts of *H. barteri*. Cardiac glycosides and catecholamine are agents of choice in treatment of congestive heart failure. Extracts from *Digitalis purpurea* and *Digitalis latana* have been used for that purpose<sup>9, 24</sup>. While some cardiac glycosides are entirely poisonous, (for example, oleanderin from *Merium oleander*), some including *Digitalis* spp. have low therapeutic index, meaning the therapeutic dose is not much lower than the toxic dose. Such glycosides cause intoxication and other complications such as gastrointestinal symptoms (nausea, vomiting, and diarrhoea), visual disturbances, neurological symptoms (headache, neuralgia, drowsiness) and cardiovascular failure and arrhythmia<sup>9, 19</sup>. Terpenoids were slightly detected only in DCM and EA extracts. Plant terpenoids exhibit various pharmacological activities such as anti-inflammatory, anticancer, antimalarial, inhibition of cholesterol synthesis, antiviral and antibacterial activities<sup>25</sup>. Terpenoidal saponins isolated from *platycodon grandiflorum* inhibited Hepatitis C virus replication<sup>26</sup>. The plant extracts did not show presence of steroids which was in agreement with<sup>5</sup>. Anabolic steroids have been observed to promote nitrogen retention in osteoporosis and animals with wasting illness<sup>14</sup>. By use of Braymer's test, tannins were revealed to be abundantly present in all the extracts except in DCM extract. Tannins are known to inhibit pathogenic fungi<sup>14</sup>; the growth of many fungi, yeast, bacteria and viruses were inhibited by tannins. Tannins

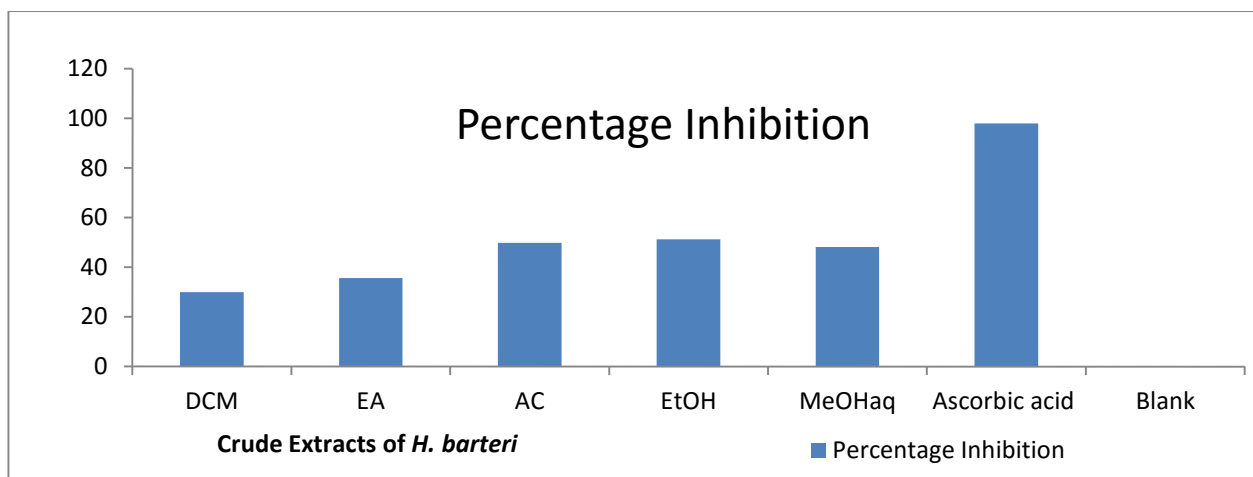


Figure 1: percentage Inhibition of DPPH Scavenging Effect by Crude Extracts

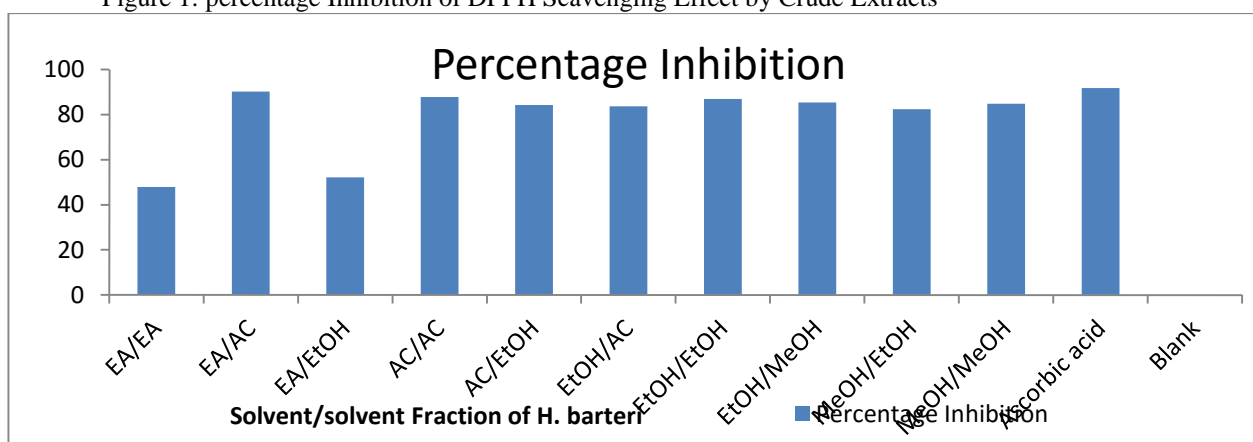


Figure 2: Percentage Inhibition of DPPH Scavenging Effect by solvent Fractions

have also been reported to exert other physiological effects such as to accelerate blood clotting, reduce blood pressure, decrease serum lipid level, produce liver necrosis and modulate immunoresponse. Plant tannins have also been shown to provide a novel therapeutic option for major factors in the induction of ulcerative colitis<sup>27</sup>. Hydrolysable tannins are potentially toxic to animals. Consumption of feeds containing high levels of hydrolysable tannins causes liver and kidney toxicity and lead to death of animals<sup>28</sup>. Anthraquinones were not detected in all of the plant's extracts. Anthraquinones are considered to be one of the most active agents in treatment of metastatic breast cancer<sup>14</sup>. Most plants containing anthraquinones possess laxative properties. The derivatives of anthraquinones present in purgative drugs may be dihydroxy phenols and trihydroxy phenols such as chrysophanol and emodins respectively<sup>9</sup>. Reducing sugars were slightly present in AC, EtOH and MeOHaq extracts. The percentage inhibition of the scavenging activities of the crude extracts of *H. barteri* on DPPH free radical was shown on Tables 2. It shows that acetone, ethanol and aqueous methanol extracts have percentage inhibition of 49.9%, 51.3% and 48.2% respectively. Compared to the standard drug, ascorbic acid which has percentage inhibition of 98% the extracts cannot be regarded as strong antioxidants let along dichloromethane and ethyl acetate

extracts. In fact methanol, ethanol and acetone have been found to be more effective in extracting antioxidant compounds from plants<sup>30</sup>. The three solvents have been found to be good in extracting phenolic compounds and flavonoids with aqueous acetone being the best. On fractionating the crude extracts, the resulting fractions (Table 3) showed improved antioxidant activities. Fractions from acetone and methanol gave percentage inhibition very close to the standard drug. However, it had earlier been reported<sup>30</sup> that, methanol has generally been found to be more efficient in extracting lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone, whereas ethanol is also a good solvent for the same purpose and safer for human consumption. Some compounds with antitumor activities were isolated based on only their antioxidant properties<sup>15</sup>. Bioassay guided fractionation of active extract of *Chorizanthe diffusa* using DPPH free radical scavenging assay led to the isolation of one novel compound (flavone) with antitumor property from ethyl acetate fractions. Table 3 shows the percentage inhibition of solvent fractions of *H. barteri* on DPPH which is quite close to that of the standard ascorbic acid. This means that they could contain bioactive components that elicit antioxidant properties and, perhaps that could

also be the reason why the plant is used traditionally to manage tumor, anemia and hemorrhage.

## CONCLUSION

The phytochemical screening of the crude extracts obtained by sequential solvent extraction of dried stem bark of *Haematostaphis barteri* showed the presence of flavonoids, cardiac glycosides and tannins as the major secondary metabolites in the extracts. The free radical scavenging properties of the extracts were determined quantitatively by the use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and result revealed that only acetone, ethanol and aqueous methanol extracts were weakly active with percentage inhibition of DPP activities of 49.9%, 51.3% and 48.2% respectively. But on purification by solvent/solvent fractionation all fractions from acetone, ethanol and aqueous methanol and some from ethyl acetate gave high percentage inhibition of DPPH activity comparable to standard antioxidant, ascorbic acid. The high antioxidant capacity of the extract fractions could be due to the presence of the phenolic compounds such flavonoids and tannins.

## REFERENCES

- Cannell, J. P. Richard (1998). How to Approach the Isolation of Natural Products, In: Cannell, J. P. Richard (Ed.). *Methods in Biotechnology: Natural Products Isolation*. Totowa, New Jersey: Humana Press Inc.
- Silva, L. Gloria; I. K- Soo Lee and A. Douglas, Kinghorn. (1998). Special Problems with the Extraction of Plants, In: Cannell Corrado, (Ed.), *Methods in Biotechnology, Natural Product Isolation*. Totowa, New Jersey: Humana Press Inc.
- Kubmarawa D., G. A. Ajoku, N. M. Nwerem and D. A. Okorie (2007). Preliminary Phytochemical and Antimicrobial Screening of 50 Medicinal Plants from Nigeria. *African Journal of Biotechnology*, 6(14): 1690 - 1696
- Rabo, E. T. and Sanusi, S. S. (2001). *An Inventory of Medicinal Plants of Nigerian Savannah*. Leviathan Books. 21- 24
- Boampong, Johnson Nyarko, Akua Afryie Karikari, Elvis, OforiAmeyau (2013). In vivo Antiplasmodial and In vitro Antioxidant Properties of Stem Bark Extracts of *Haematostaphis barteri*. *Asian Journal of Tropical Biomedicine*, 5(6): 446 – 450
- Wurochekke Abdullahi Usman Bello Huseini, Yusuf Hajara Balarabe and Mahmoud Suleiman Jada (2014). In vitro Trypanocidal Activity of Aqueous Stem Bark Extract of *Haematostaphis barteri* Against Trypanosomes *congelense* and *Trypanosoma brucei brucei*. *Scholars Academic Journal of Pharmacy*, 3(1): 38 - 42
- Seidel, Veronique. (2006). Initial and Bulk Extraction, In: Sarker, D. Satyajit; Zahid Latif and A. I. Gray, (Eds.). *Methods in Biotechnology: Natural Products Isolation*. Vol. 20 2<sup>nd</sup> Edition. Totowa, New Jersey: Humana Press Inc.
- Sofowora, Abayomi. (2008). *Medicinal Plants and Traditional Medicine in Nigeria*. Ibadan: Spectrum Books Ltd.
- Evans, C. Williams (2009). *Trease and Evans Pharmacognosy*, 6<sup>th</sup> Edition. China: Saunders Elsevier
- Tiwari Prashant, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur, (2011). Phytochemical Screening and Extraction. A review. *International Pharmaceutica Scientia*. 1(1): 98- 106
- Soni Anjali and Sheetal Sosa, (2013). Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. *Journal of Pharmacognosy and Phytochemistry*. 2(4): 22- 29
- Marinova, G. and V. Batchvarov (2011). Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH. *Bulgarian Journal of Agricultural Science*, 17: 11- 24
- Sampietro A. Diego, Cesar A. N. Catalan and Marta A. Vattuone (editors.). *Isolation, Identification and Characterization of Allelochemicals/Natural Products*. New Hampshire: Science Publishers
- Lalitha P. Thamaraiselvi and P. Jayantha (2012). Preliminary Studies on Phytochemicals and Antimicrobial Activity of Solvent Extracts of *Eichornia crassipes* (Mart.) Solms *Asian Journal of Plant Sciences and Research*. www.pelagiaresearchlibrary.com
- Chang, L. C., and A. D. Kinghorn, (2001). Flavonoids as Cancer Chemopreventive Agents, In: Tringali, Corrado. *Bioactive Compounds from Natural Sources*. London: Taylor and Francis
- El-Alfy S. Taha, Hamidu M. M. El-Gohary, Nadia M. Sokkar, Mohammed Hosney and Dalia A. Al-Mahdy (2011). A New Flavonoid C-glycoside from *Celtis australis* L. and *Celtis occidentalis* L. Leaves and Potential Antioxidant and Cytotoxic Activities. *Scientia Pharmaceutica*, 79(4): 963 - 975
- Alzeer Jawal, Balayeshwanthe R. Vummidi, Rami Arafeh, Waleed Rimawi, Hatem Saleem and Nathan W. Luedtke (2014). The Influence of Extraction Solvents on the Anticancer Activities of Palestinian Medicinal Plants. *Journal of Medicinal Plant Research*, 8(8): 408 - 415
- Mata, R.; J.-F. Rivero-Cruz and D. Chavez, (2001). Flavonoids as Cancer Chemopreventive Agents, In: Tringali, Corrado. *Bioactive Compounds from Natural Sources*. London: Taylor and Francis
- Pengelly, Andrew (2004). *The constituents of Medicinal Plants: An Introduction to the Chemistry and Therapeutics of Herbal Medicine*, 2<sup>nd</sup> Edition. Australia: Allen and Unwin
- Kalase, Vaibhav and Varsha Jadhav (Rathod) (2013). Preliminary Phytochemical and Antibacterial Studies on Leaf and Bark of *Holigrarna grahamii* (Anacardiaceae). *Journal of Applied Pharmaceutical Science*, 3(6): 111 – 113
- Edrah, Salem, Fawzy Alafid, Ashok Kumar (2013). Preliminary Phytochemical Screening and Antibacterial Activity of *Pistacia atlantica* and *Prunus*

- persica Plants of Libyan Origin. *International Journal of Science and Research*, 6(14)
22. Ajayi, I. A., Ajibade, O. and Oderinke, R. A. (2011). Preliminary Phytochemical Analysis of Some Plant Seeds. *Research Journal of Chemical Sciences*, 1(3): 58 - 62
23. Sharma Veena and Ritu Paliwal (2013). Isolation and Characterization of Saponins from *Moringa oleifera* (Moringaceae) Pods. *International Journal of Pharmaceutical Sciences*, 5(1): 180-183
24. Rashmika, Patel and Patel Manish P. (2012). Cardiotoxic Activity of Isolated Cardiac Glycoside from the Fruits of *Corchorus estuans* Linn. *International Research Journal of Pharmacy*, 3(7): 239 - 242
25. Wadood Abdul, Mehreen Ghufuran, Syed Babar Jamal, Mohammed Naeem, jmal Khan, Rukhsana Ghaffar and Asnad (2013). Phytochemical Analysis of Medicinal Plants Occuring in Local Area of Mardan. *Biochemistry and Analytical Biochemistry*. <http://dx.doi.org/10.4172/2161-1009.1000144>
26. Kim Jong-Woo, Sang Jin Park, Jong Hwan Lim, Jae Won Yang, Lung Chuel Shin, Sang Wook Lee, Joo Won Suh and Soon B. Hwang (2013). Triterpenoid Saponins Isolated from *Platycodon grandiflorum* Inhibits Hepatitis C Virus Replication. *Evidence Based Complementary and Ulternative Medicine* 2013/2013. <http://dx.doi.org/10.1155/2013/560417>
27. Clinton Catherine (2009). Plant Tannins: a Novel Approach to the Treatment of Ulcerative Colitis. *Natural Medicine Journal*, 1(3): 1 – 4
28. Makker P. S. Harinder, P. Siddhuraju and Klause Becker (2007). *Plant Secondary Metabolites*. Totowa, New Jersey: Humana Press
29. Dai, Jin and Mumper, J. Russell (2010). Plant Phenolics: Extraction, Analysis and their Antioxidant and Anticancer Properties. Review. *Molecules*. 15: 7313-7352