International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 641-647
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Phytochemical Screening, Isolation of Flavonoids from *Hellaenia speciosa* (J.Koenig) S.R Dutta and Study of Its Antibacterial Activity *In Vitro*

[1]Debasish Singha Roy, [1]Somnath De, [1]Subhasish Maity, [1]Writtik Maity and [2]Dulal Chandra Das*

[1]Department of Biotechnology, Panskura Banamali College (Autonomous), West Bengal, India.

[2]K. D College of Commerce and General Studies, Midnapore, West Bengal, India.

Received: 14 Mar 2019 / Accepted: 16 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** pbcsomnath@gmail.com

Abstract

Hellenia speciosa an important herbal to the tribal, not founded as vivid day up till now. Except few fragmentary findings no investigation in respect to phytochemical screening. And antibacterial activity of the present day have not been done keeping this point in mind phytochemical screening and anti-bacterial activity recently done against four bacteria *(E. coli, S.typhi, S. aureus, K.pneumoniae)* using Acetone, Methanol, Petroleum Ether as organic solvent. Flavonoids as marker of phytochemicals identified for best results where Methanol extracts for *S.typhi* and Acetone extract for *S.aureus* showed excellent results rather than extract in Petroleum Ether.

Keywords

Hellenia speciosa, flavonoids, antibacterial activity.

*****

INTRODUCTION

Plants synthesize many potent phytochemicals which considered as best remedy for the treatment of many pathogenic and non-pathogenic disorders in human beings. Different parts of the body contain significant amounts of bioactive components[1]. These components are mainly secondary metabolites and their beneficial effects come from the combined effect of them. Particular group of plants possess a particular chemical which is specific for that group and taxonomically distinct from the others. The synthesis of these chemicals also depends upon environmental condition like scarcity of water, salinity, high temperature etc. In the arid and semi-arid zones plants produce various types of secondary metabolites which are helpful for their existence[2].

Flavonoids should have great antioxidant properties and can play versatile protective role against oxidative stress [3-8]. Most of the cases plants produces very important secondary compounds like polyphenols, flavonoids etc[9]. Flavonoids are generally plant pigments found in some different

vegetables, flowers, fruits, stems, root and leaves [10]. According to online food database "Phenol-Explorer" different onions, tea, grape fruits, red-wine, citrus fruits, cocoa beans are the chief source of flavonoids [11-13]. To save the next generation of human beings polyphenols must have to take in their regular food chart and food habit as suggested by different meta-analysis [14]. Different plant polyphenols have antioxidant and anti-microbial activity which can control many fatal diseases. They can reactive phase-II metabolism and produce sulphated, methoxylated and glucuronidated compounds for destroying free radicals inducing apoptosis, inhibiting cell proliferation and ageing [15,16]. Flavonoids are one kind of polyphenols that have low molecular weight and have bioactive properties [17-19].

Until now more than 2000 flavonoids have been reported in the world, all are collectively known as Vitamin P, isolated from many plants like Jatropa, Cassia, Quercus etc. Generally synthetic drugs are used to control the various diseases. Synthetic drugs create health hazards, due to which modern day science now trying to find out phytochemicals as phytomedicines or as herbals to repair or to recover that damages. Flavonoids are such a type of phytomedicines may be used alternatively as substitutes. Investigation is going on rapidly throughout the world. The antibacterial activity of flavonoid compounds has very potent mode of action against pathogenic microorganisms [20].

In the present investigation attempts have been made to isolate the flavonoids from *Hellenia speciosa* and screening the antibacterial activity against some human pathogens.

MATERIALS AND METHODS

Selection of plant material

Most of the villagers and tribal people used the *Hellenia speciosa* for their common diseases regularly. On that context we have chosen the material as experimental tool. It is collected in the month of October, 2018 form keshpur Paschim Medinipur district (Latitude- 22°33'16.2" north, Longitude- 87°27'40" east, Altitude- 41 meters from mean sea level), West Bengal, India and it is available in any season of year.

Description and Identification

Plant material have fleshy stem, big leafs, white flower and rhizome like root. Plant material are having been identified from CNH (Central National Herbarium), BSI (Botanical Survey of India), Shibpur, Howrah; specimen no- 'DSR-39'.

Surface Sterilization

A healthy, disease free fresh whole plants were selected to extract the different solvents and wash with distilled water. Then 0.1% mercuric chloride was added to the whole plants for 20 seconds to done the surface sterilization. Again three times wash with distilled water.

Plant material extraction

After surface sterilization of whole plant materials of *Hellenia speciosa* cut into small pieces, kept it few days for drying under shade. Then the air dried plant material grinded into powder. The powdered material was extracted with acetone, ethanol, methanol, chloroform, petroleum ether, hexane and distilled water using soxhlet apparatus. About 10 grams of powder was loaded in soxhlet extraction unit and exhaustively extracted using 100ml of solvents such as acetone, ethanol, methanol, chloroform, petroleum ether, hexane and distilled water respectively at 60°C for 12 hours. Thereafter, it was filtered with the help of Whatman No-1 filter paper and use for various phytochemical analysis.

Phytochemical Screening

Phytochemical analysis of the test sample was carried out according to standard methods [21,22].

Test for Alkaloids

The alkaloids test was performed by the help of Wagner's reagent [100 ml of water contain 2 g of potassium iodide and 1.27 g of iodine]. The different solvent extracts of plant were added to this reagent and observed for the formation of reddish-brown precipitate.

Tests for Carbohydrates

2ml of various plant extracts were added with few drops of Molisch's reagent. Then the addition of concentrated H_2SO_4 (2ml) to the test tube and allowed to wait for two-three minutes. After few minutes later a red or dull violet colour was formed of the two layers it indicates the positive test.

Test for Cardiac glycosides

In a test tube 5ml of each plant extract was taken and glacial acetic acid (2ml) used to treat them and addition of few drops of ferric chloride solution. Then carefully sulphuric acid (1ml) was added to the tested solution. At the junction of the two solutions a brown ring is appeared which indicates the presence of deoxy-ribo sugar characteristic of cardenolides. After that greenish ring may form to indicate the presence of Cardiac glycosides.

Test for Flavonoids

A portion of different crude extract of plant was added of 5ml of dilute ammonium solution, followed by addition of concentrated H_2SO_4. After that a

yellow coloration may formed in each of the plant extract indicates the presence of flavonoids.

Test for Phenols

1ml of various solvent extracts of plant kept in to the different test tubes, 2ml of distilled water and few drops of 10% ferric chloride solution was added. Then blue or green colour was formed which indicate the presence of phenols.

Test for Phlobatannins

2 ml of each plant extract was boiled with 1ml of 1% aqueous hydrochloric acid to deposition of a red precipitate, which can indicate the presence of phlobatannins.

Test for Amino acids and Proteins

To perform the presence of amino acids and proteins few drops of Ninhydrin solution treated with different plant solvent extracts and it placed with boiling water bath for two 2 minutes and observed the formation of purple colour.

Test for Saponins

The formation of foam if 5ml of distilled water mixed with 2ml of different plant extract in a test tube and it was shaken vigorously after that addition of few drops of olive oil indicate the presence of saponins.

Test for Tannins

In a test tube 1ml of various plant extract was taken and then addition of 1ml of 0.008 M potassium ferric cyanide. After that, 1ml of 0.02 M ferric chloride containing 0.1 N HCL was added and observed for blue -black coloration.

Test for Terpenoids

To perform the terpenoids test in a test tube different plant extract was kept and added concentrated H_2SO_4 and chloroform. After few minutes later if a reddish brown precipitate formed then it shows the positive test.

Test for Quinones

Formation of yellow precipitate if concentrated HCL added to the different plant extract indicates the presence of quinone.

Bacterial strains and culture conditions

The whole plant extracts of *Hellenia speciosa* were screened against three pathogenic bacteria collected from the Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The test organisms were *Escherichia coli* (MTCC 1687), *Staphylococcus aureus* (MTCC 3160), *Salmonella typhi* (MTCC 98). The nutrient agar slant culture was maintained at 37ºC and every 48 hours of transferring.

Agar well diffusion method

The different solvent extracts of *Hellenia speciosa* was used to determine the antibacterial activity by agar well diffusion method. Muller-Hinton agar (MHA) was used to perform this experiment. Twenty microliters of each extract were poured into each well and plates were incubated at 37 °C for 24 h. After 24hrs the zone of inhibition was measured in millimetre and the antibacterial results are taken[23].

Flavonoid extraction

Hellenia speciosa whole plant cuted in small pieces, kept it few days dried under shade. Then the air dried plant material grinded into powder. Then this powder material was used further for flavonoid extraction procedure. 100gm of whole plant powder of *Hellenia speciosa* was immersed in 500ml ethanol (HPLC grade; Merck, India) for 24 hrs at room temperature on a magnetic stirrer [24]. Whatman No.-1 filter paper was using to filter the mixture. 300ml ethanol was added to the remaining residue and again the whole process was repeated. After filtration was completed, the filtrates were mixed with 100ml 1% lead acetate solution, stirred it for 4 hrs for complete precipitation, filtrate and filtered was taken. Then 30ml HCL and 250ml acetone mixture (Merck, India) was taken and mixed with the filtrate. At 4ºC the total mixture solution was stored. In this way a deep brown colour precipitate was formed and it was further used a rotary evaporator under reduce pressure to get the actual unknown identified flavonoid compound.

Chemical screening of flavonoid

Different chemical tests were done the dry extract of plant to investigate the presence of different phytoconstituents [25, 26].

Flavonoids: - The chemical screening was widely used to detect the flavonoids presence in the plant extract. The alcoholic plant extracts mixed1% KOH solution and observes the yellow colour formation.

Phenolic compound: - 1% of ferric chloride 1-2 drops was added to the 5ml of plant extract, then appear a blue-green colour which clearly indicated the presence of phenolic compounds.

Double bond test: - Identify the presence of double bond character by the use of KMnO4 reagent, brown solution formed indicates the presence of double bond.

Aldehyde & ketone test: - When the extract compound mixed with 2, 4, dinitrophenyl hydrazine then it shows yellow precipitate, which indicate the extracted compound has aldehyde and ketone groups.

Antibacterial screening of isolated flavonoids compound.

The isolated unknown flavonoids compound used to investigate the antibacterial activity of most common human pathogens like *Escherichia coli, Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumonia*e, by Agar Well Diffusion method[27].

Measuring the triplicate zone of inhibition was recorded to understand the antibacterial screening assay.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Different tribal communities totally depend upon medicinal plants for their healthcare because it has great significance. The plant extracts of the experimental tool phytochemicaly analysed and different biochemical constituents have been proved their existence. After careful analysis of the *Hellenia specious* plant extracts revealed the presence of phytochemicals, such as proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, phlobatannins, terpenoids and alkaloids. During my present study, preliminary phytochemical analysis revealed a large amount of flavonoids, phenolic compounds, terpenoids and cardiac glycosides present in different whole plant extract of *Hellenia speciosa*.

The phytochemical analysis of whole plant of *Hellenia speciosa*, represents interesting results where flavonoid present in all the tested extracts where as terpenoids, tannins and cardiac glycosides are totally absent shown in **Table 1.** The positive phytochemicals of this experiment lead to investigate further their pharmacological activities. In future, detail research on this plant can reveal a new era of phytochemistry and provide more active phytoconstituents for pharmacological significance. It should be finding new generations of drug that may be treat the very serious problems of diseases.

Table1: Preliminary phytochemical screening of *Hellenia speciosa*

SL NO.	PHYTOCHEMICALS CONSTITUENTS	ACETONE EXTRACT (A.E)	METHANOL EXTRACT (M.E)	PETROLIUM ETHERE (P.E)
1	Alkaloids	-	+	+
2	Cardiac glycosides	-	-	-
3	Carbohydrates	+	+	+
4	Flavonoids	+	+	+
5	Phenols	+	+	+
6	Saponins	-	-	-
7	Sterol	-	+	+
8	Tannin	-	-	-
9	Terpenoids	-	-	-
10	Quinines	-	+	-
11	Protein	+	+	+

N.B + = Positive, - = Negative. A.E = Acetone extract, M.E = Methanol extract, P.E = Petroleum ether extract.

Antibacterial activity

Experimental findings reveal *Hellenia speciosa* is the best herbal remedy to control specially *S. typhi, E. coli, S. aureus*. The phytochemical constituents which are responsible for many pharmacological activities, may be useful for the evolution of pharmaceutical and for the therapy of ailments. This is the first ever experimental findings of antibacterial activity as well as demonstration of any biological properties in the globe but deserve further investigation to develop new medicine that may help in combating several diseases in tropical countries to some extent. Further detail and accurate studies on this plant will explore a dynamic field of bioactive compound which is help to develop new antibacterial medication. After doing the experiment it is revealed that methanol extract is much more crucial to control *S.typhi* and acetone extract is highly specific to control *S.aureus* whereas petroleum ether extract has less efficiency to release the essential phytoconstituents, antibacterial activity shown in **Table2, Figure1 and Figure2.**

Table 2: Antibacterial activity of different solvent whole plant extracts of *Hellenia speciosa* against human enteric pathogen

Whole plant extract	*Escherichia coli* (MTCC-1687)	*Staphylococcus aureus* (MTCC-3160)	*Salmonella typhi* (MTCC-98)
Acetone (A.E)	27.66mm	33.00mm	26.66mm
Methanol (M.E)	26.00mm	28.00mm	28.33mm
Petroleum Ether (P.E)	12.66mm	-	14.00mm

A.E = Acetone extract, M.E = Methanol extract, P.E = Petroleum ether extract, Values are mean of three replicates, ± values represent SD.

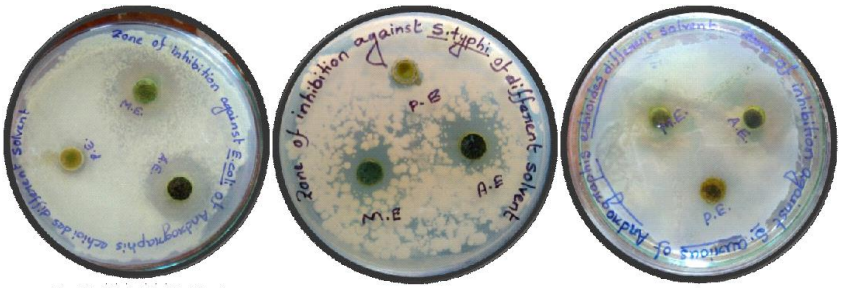

Figure1: Agar well diffusion method of different solvent whole plant extracts of *Hellenia speciosa* against human enteric pathogens.

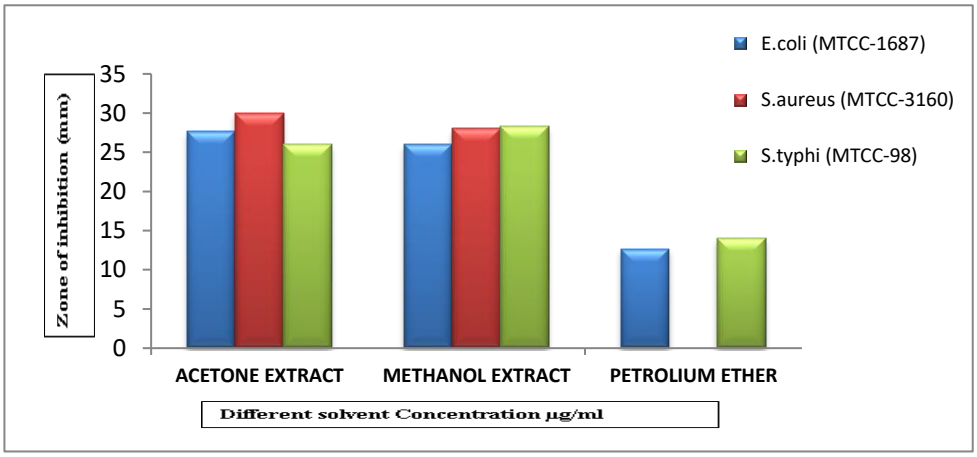

Figure2: Antibacterial activity of different solvent whole plant extracts of *Hellenia speciosa* against human enteric pathogens. Physiochemical properties of the isolated flavonoid compound

The isolated unknown compound from was used to investigate their physiochemical properties. We got the plant material which is crystal like appearance and deep brown in colour. This unknown compound revealed the positive results of presence of phenolic -OH group, un-saturation and existence of flavonoid compound **(Figure 3).** Some physiochemical properties of the compound have shown in the **(Table 3).**

Table 3: Physical and chemical characteristics of the isolated compound

Test	Flavonoid
Melting point	137°C
Solubility test	Soluble in ethanol, methanol, chloroform, acetone but insoluble in water.
Description	Red-brown crystal

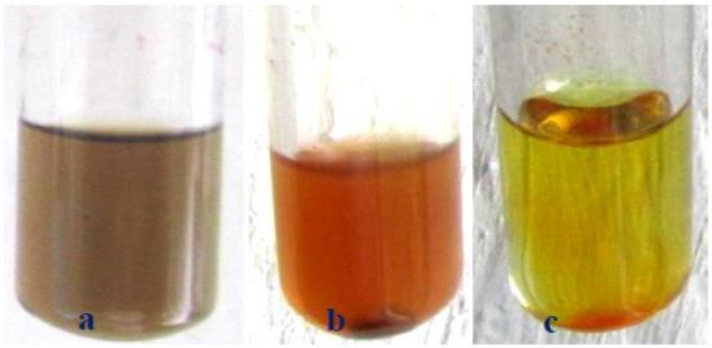

Figure 3: Functional group tests a) presence of phenolic -OH groups. b) Presence of unsaturation and c) Yellow precipitation during flavonoid test.

Antibacterial screening of isolated flavonoids compound.

It shows very high antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae*. The compound has comparatively low antibacterial activity against *Escherichia coli* results shows in **Table4 and Figure4 and 5.**

Table 4: Antibacterial activity of Agar well diffusion method by isolated pure flavonoid compound from *Hellenia speciosa against* human enteric pathogens.

Antibacterial activity of Agar well diffusion method in different concentration			
Microorganisms	**40 µg/ml**	**80 µg/ml**	**100 µg/ml**
Escherichia coli	9.2 mm	13.67 mm	21 mm
Staphylococcus aureus	14 mm	18 mm	23.34 mm
Salmonella typhi	6 mm	13.33 mm	23.03 mm
Klebsiella pneumoniae	9.56 mm	14.34 mm	22.67 mm

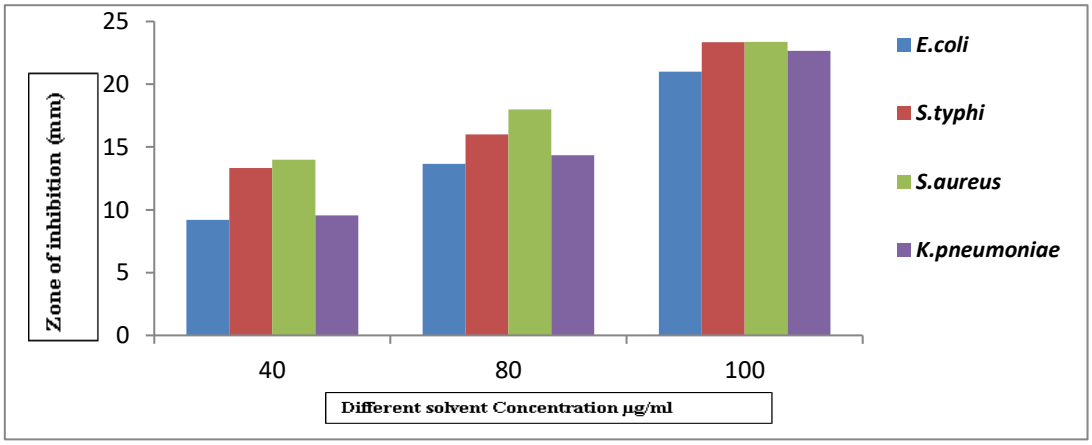

Figure 4: Antibacterial activity of Agar well diffusion method by isolated pure flavonoid compounds from *Hellenia speciosa* against human enteric pathogens.

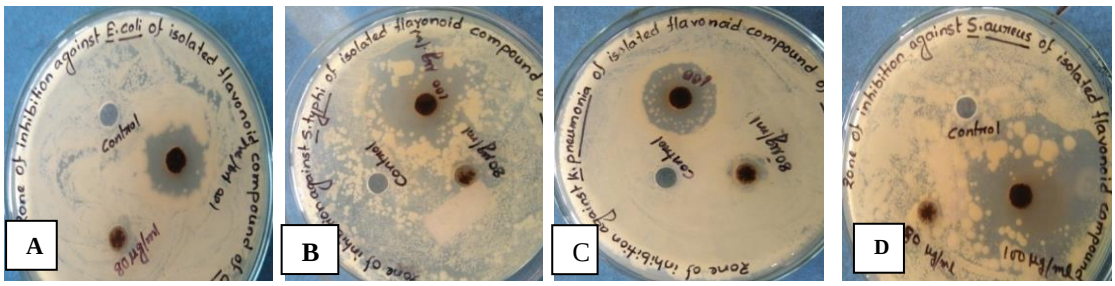

Figure 5: Antibacterial activity of the isolated flavonoid compound clear zone of inhibition found against A) *Escherichia coli* B) *Salmonella typhi* and C) *Klebsiella pneumoniae* D) *Staphylococcus aureus*

CONCLUSION

S.typhi and *S.auerus* both the pathogen survive in the tropical countries like India. Infection in the GIT as well as in the skin of human beings is mainly caused by these two pathogens. *Hellenia speciose* is a common herbal in India in spite of that the Indian people mostly suffering from enteric disease and large boils called Carbuncle in the skin, mostly found during summer season. People of India may easily use it and can prevent the infection. Future research upon this plant may open a new avenue for the non-conventional medical research.

ACKNOLEDGEMENT

Authors are thankful to Panskura Banamali College, Karry Mullis Molecular Biology Laboratory (Sponsored by BOOST, Govt. of West Bengal, India) for providing facilities and financial support for the completion of this research work. Authors are thankful to CNH, BSI, Shibpur, Howrah, for identification and authentication of the plant

species. We are very much thankful to Prof.(Dr) Dulal Chandra Das for his huge and tremendous help and support.

REFERENCES

1. Gordon, MC., David, JN. Natural product drug discovery in the next millennium. Pharm. Biol. 2001;39 :8-17.
2. Wink, M. Introduction Biochemistry, role and biotechnology of secondary products. In: M Wink, Ed, Biochemistry of Secondary Product Metabolism. CRC Press, Boca Ratom, FL.1999; 1-16.
3. Pollastri, S., Tattini, M. Flavonols: old compounds for old roles. Annals of Botany 2011; 108:1225-1235.
4. Quintans, JSS., Antoniolli, AR., Almeida, JRGS., Santana-Filho, VJ., Quintans-Junior, LJ. Natural products evaluated in neuropathic pain models – A systematic review. BCPT.2014; 114:442-450.
5. Legette, L., Karnpracha, C., Reed, RL., Choi, J., Bobe, G., Christensen, JM. Human pharmacokinetics ofxanthohumol, an anti-hyperglycemic flavonoid from hops. MolNutr Food Res. 2014; 58(2):248-255.
6. Kay, CD., Hooper, L., Kroon, PA., Rimm, EB., Cassidy, A. Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products. MolNutr Food Res.2012;1-12.
7. Van Dam, RM., Naidoo, N., Landberg, R. Dietary flavonoids and the development of type 2 diabetes and cardiovascular diseases: review of recent findings. CurrOpin Lipidol.2013; 24:25–33.
8. Arts, ICW., Hollman, PCH. Polyphenols and disease risk in epidemiologic studies. Am J ClinNutr. 2005; 81:317–325.
9. Mullie, P., Clarys, P., Deriemaeker, P., Hebbelinck, M. Estimation of daily human intake of food flavonoids. Plant Foods Hum Nutr. 2007; 62: 93-98.
10. Neveu, V., Perez-Jime´nez, J., Vos, F., Crespy, V., Du Chaffaut, L., Mennen, L. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database.2010; 1-9.
11. Peterson, JJ., Beecher, GR., Bhagwat, SA., Dwyer, JT., Gebhardt, SE., Haytowitz, DB. Flavanones in grapefruit, lemons, and limes: A compilation and review of the data from the analytical literature. J Food Comp Ana. 2006; 19:74–80.
12. Shrime, MG., Bauer, SC., McDonald, AC., Chowdhury, NH., Coltart, CEM., Ding, EL. Flavonoid-Rich Cocoa Consumption Affects Multiple Cardiovascular Risk Factors in a MetaAnalysis of Short-Term Studies. J Nutr. 2011;141: 1982- 1988.
13. He, FJ., Nowson, CA., Lucas, M., MacGregor, GA. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Human Hypertention.2007;21:717-728.
14. Wang, X., Ouyang, YY., Liu, J., Zhao, G. Flavonoid intake and risk of CVD: a systematic review and meta – analysis of prospective cohort studies. British J Nutr.2014;111:1-11.
15. Adedapo, AA., Jimoh, FO., Koduru, S., Afolayan, AJ., Masika, PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Comp Alt Med.2008; 8:53.
16. Mandalari, G., Bennett, RN., Bisignano, G., Trombetta, D., Saija, A., Faulds, CB. Antimicrobial activity of flavonoids extracted from bergamot (Citrus bergamiaRisso) peel, a byproduct of the essential oil industry. JApplMicrobiol.2007;103:2056-2064.
17. Fernandez, SP., Wasowski, C., Loscalzo, LM., Granger, RE., Johnston, GAR., Paladini, AC., Marder, M. Central nervous system depressant action of flavonoid glycosides. European Journal of Pharmacology.2006; 539: 168-176.
18. Heim, KE., Tagliaferro, AR., Bobliya, DJ. Flavonoids antioxidants: Chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry.2002; 13: 572-584.
19. Hollman, PCH., Katan, MB. Dietary Flavonoids: Intake, Health Effects and Bioavailability. Food and Chemical Toxicology. 1999; 37: 937-942.
20. Cushnie, TPT., Lamb, AJ. Antimicrobial activity of flavonoids.Int. J. Antimicrob. Agents.2005;26: 343–356.
21. Shrime, MG., Bauer, SC., McDonald, AC., Chowdhury, NH., Coltart, CEM., Ding, EL. Flavonoid-Rich Cocoa Consumption Affects Multiple Cardiovascular Risk Factors in a MetaAnalysis of Short-Term Studies". J Nutr. 2011;141: 1982- 1988.
22. He, FJ., Nowson, CA., Lucas, M., MacGregor, GA. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Human Hypertention. 2007; 21:717-728.
23. Khan, AV., Ahmad, QU., Shukla, I., Khan, AA. Antibacterial efficacy of Bacopa monnieri leaf extracts against pathogenic bacteria. Asian Biomed.2010; 4:651-655.
24. Okogum, JL. Methods of medicinal plant extract preparation. National institute for pharmaceutical research and development (NIPRD). Iddu Abuja, Negeria.2000.
25. Harborne, JB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd Ed. Chapman and Hall, Ltd. London, 13th Ed. 1973; 37-89.
26. Harborne, JB. Phytochemical methods.2nd Ed., Chapman and Hall, New York, USA. 1984.
27. Mandalari, G., Bennett, RN., Bisignano, G., Trombetta, D., Saija, A., Faulds, CB. Antimicrobial activity of flavonoids extracted from bergamot (Citrus bergamia Risso) peel, a by-product of the essential oil industry. J Appl Microbiol.2007;103:2056-2064.