chemistry proceedings

MDPI

Proceeding Paper

Cumulative Phytochemical Analysis And Identification Of Drug Lead Compounds From Medicinal Plant Extracts†

Sandeep Waghulde1,* M. K. Kale2 and V. R. Patil1

[1] 1 Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, Dist: Jalgaon; Maharashtra, India

2 Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Institute, Karjat, Dist: Raigad; Maharashtra, India

*Correspondence: sandeepwaghulde@yahoo.com

† Presented at the 25th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2021; Available online: https://ecsoc-25.sciforum.net/.

Abstract:

Secondary metabolites found in the medicinal plants play important role in curing different diseases and used as important raw materials for the manufacturing of traditional and modern medicine. These medicinal plants like Annona reticulata with Allium sativum, Allium fistolisum and Brassica oleraceae reduces various risk factors associated with several diseases. It has been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and LDL, increase antioxidant status, and inhibit angiotensin converting enzyme. It also reduces cholesterol, inhibits platelet aggregation, reduces blood pressure, and increases antioxidant status. Therefore, our aim was to compare the different secondary metabolites present in the crude extracts of aqueous and methanolic. Phytochemicals screening revealed the results that alkaloids, reducing sugar, flavonoids, glycosides, cardiac glycosides, tannin and phenolic compounds, saponins, amino acid & triterpenoids It indicated that bioactive components are present in these plants that could be accounted for its pharmacological effects. Thus, the results support the uses of these plant species in traditional medicine.

Keywords: Annona reticulate, Allium sativum, Allium fistolisum, Brassica oleraceae Phytochemical components, Medicinal plants.

Academic Editor: Julio A. Seijas

Published: 15 November 2021

chemistry proceedings

Introduction:

Natural products, including plants, animals and minerals have been the basis of treatment of diseases from time immemorial. History of medicine dates back practically to the existence human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community.1

Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. The World Health Organization has estimated that 80% of the earth's inhabitants rely on traditional medicine for their health care needs, and most of this therapy involves the use of plants extracts or their active components. Therefore, therapeutic approach of several traditional medicines is rather more holistic. Majority of fundamental concepts of their medicinal systems still cannot be explained using modern tools.2

Medicinal plants sector has traditionally occupied an important position in the sociocultural, spiritual and medicinal area of rural and tribal lives of India. The global thrust areas for drugs from medicinal plants include disease conditions, whose incidence is unavailable or unsatisfactory. International market of medicinal plants is over US $ 60 billion per year, which is growing at the rate of 7% annually.3

Annona reticulata Linn one of the traditionally important plant used for the treatment of various ailments.4 It belongs to family Annonaceae.5 The synonyms of plant are Ramphal, Bullock's heart and Custard apple.6 Near about 119 different species of the Annona genus (Annonaceae) are identified among which most of them are shrubs and trees. Traditionally the plant extract is used for the treatment of diarrhoea7-8 and pediculosis.9

Allium sativum commonly known as garlic belongs to family Amaryllidaceae. Name of garlic is poondu in Tamil, veluthulli in Malayala, vellulli in Telugu, rasoon in Bengali, lasan in Gujrati, lasun in Marathi, lassan in Punjabi & lassun in urdu. Its close relatives include the onion, shallot, leek, chive10 and Chinese onion11. With a history of several thousand years of human consumption and use, garlic is native to Central Asia and has long been a common seasoning worldwide. It was known to Ancient Egyptians has been used both as a food and as a traditional medicine12-13.Garlic one of the oldest plants used throughout history for both culinary and medicine ranks the highest of all the herbal remedies consumed for its health benefits. The bulbs of the plant have been used in many parts of the world as a stimulant, antiseptic, anthelminthic, antihypertensive, carminative, diaphoretic, expectorant, diuretic, antiscorbutic, aphrodisiac and antiasthmatic and for the relief of rheumatic pains14. Physicians prescribed the herb during the middle ages to cure deafness and the American Indians used garlic as a remedy for earaches, flatulence, and scurvy. Recent

research revealed that garlic is not only beneficial as a medicinal plant, but it can be used as a repellent to some plant pests and diseases15-16.

A. sativum (garlic) and A. cepa (onion) have a variety of pharmacological effects including chemopreventive activity and tumor cell growth inhibition17-18. The antioxidant activity of Allium species is due to a variety of sulphur-containing compounds and their precursors, but it is also related to other bioactive compounds: polyphenols, dietary fibers, microelements.
The major flavour component of garlic is a thiosulphinate called allicin, which is duly formed when the garlic tissue is damaged due to the hydrolysis product of S-allyl cysteine sulphoxide (alliin) which is specifically produced by the enzyme allinase.
A. fistulosum L. (Spring/ Welsh onion) is one of the cultivated species of Allium. Welsh onion is a perennial species originated from Eastern Asia. Its leaves have nutritional value, and they can be fresh consumed all over the year, still green over the winter.
The medicinal properties, especially antifungal and antioxidant were determined, and they are due to sulphur containing compounds, flavonoids, fatty acids19-21. To increase our understanding of the pharmacological and nutraceutical properties of Allium species, further comprehensive study of its nutrients, especially allicin, polyphenolic compounds and phytosterols, is required.

Cruciferous vegetables are "vegetables of the Brassicaceae family also called as cruciferae". Brassica vegetables are greatly regarded for their nutritional value, they are rich source of vit. C, soluble fiber as well as contain multiple nutrients and phytochemicals. Phytochemicals are the compounds derived from plants hypothesized to be responsible for much of the disease protection in our body they are present in diet high in fruits, vegetables, cereals and plant based beverages 22.

Red cabbage is the member of Brassicaceae family. It is a cool season cruciferous vegetable. Red cabbage (Brassica oleracea var. capitata f. rubra) is type of cabbage, also well-known as purple cabbage, blue kraut, or red kraut and is widespread in the Mediterranean region23.
The principle "bioactive components of red cabbage are isothiocyanates, vitamins A, B, C and anthocyanins"24-26. Anthocynanins, a natural pigment present in Red cabbage, were found to have the strongest antioxidant power of 150 flavonoids27. They are water soluble pigments it can be red, blue or purple depending on the pH. Along with the "substances that seem to be responsible for the biological activities of red cabbage, are polyphenols"28.
Red Cabbage contains many bioactive substances. Therefore, it has greatly therapeutic importance for humans. Red cabbage is an excellent source of both types of fibers. Insoluble fibers help to prevent from constipation and reduce colorectal risk. Soluble fiber present in red cabbage helps to lower blood sugar and blood cholesterol therefore helps in reducing risk of heart diseases and diabetes.29. "Many studies conducted on red cabbage extract reveal its ability to suppress the oxidative stress in vivo30-31. Park YJ. et al.32 has reported anticancer properties in red cabbage". Aml FM. Morsy et al33 have concluded in their study that red cabbage has a protective action against hepatocellular carcinoma in rats, thus this study suggest that increased dietary intake of red cabbage may be beneficial for patients with liver cancer as a

preventive measure. Several studies have stated anti-inflammatory34, analgesic35, anti-bacterial effects36 and antidiabetic37 effects of red cabbage.

Material & Method

Plant material

The fresh leaves of A. reticulata and Allium sativum (bulbs) were collected from regions of Karjat Dist-Raigad, Maharashtra, India in October 2019. Spring onion and Red cabbage were collected from regions of Kalyan Maharashtra, India. Plant materials were authenticated at "The Blatter Herbarium" -St. Xavier's College, Mumbai.

Method

After identification and authentication of the plant, leaves of the plant were collected for the experimental process. The leaves were shade dried, made into coarse powder and the powdered material was initially defatted with petroleum ether and then subjected to cold maceration process for 60h using same proportions of mixture of methanol and water as solvent to prepare hydro-alcoholic extract of Annona reticulata leaves (percentage yield 20.5% w/w with respect to dried powder). The extract was filtered and concentrated by rotary evaporator. For the preparation of different fractions method was used.

The raw Allium sativum was sliced, crushed, dried in air and then pulverized to powder. The extraction was performed by soaking 100g of the pulverized garlic in 600ml of distilled water for 24 hours, the residue and the filtrate were obtained by filtering the soaked garlic (Allium sativum) using Whatman No. 1 filter paper. The residue was dried on a cardboard paper and the filtrate was obtained as extract.

Random samples of spring onion's samples were collected from local retail markets of Karjat, Maharashtra, India. Samples were then washed thoroughly with tap water to remove dust and dirt particles. Afterwards, the outer skin of the samples were removed and then divided into small sections and they were placed into hot oven, for drying at 40°C. The dried samples were grinded into fine powder by using a grinder and then were put in glass bottles.

Ten grams of spring onion's powder were soaked in 100 mL of methanol and water, respectively. The prepared samples were shake using orbital shaker for 7 hrs followed by centrifugation for 15 min at 7000 rpm. The extracts were then filtered using vacuum filtration assembly.

Red cabbage leaves were shade dried followed by hot air oven drying at 50° centigrade and then ground to a fine powder and stored in air tight container for the analysis. Fresh red cabbage leaves were grinded in the mixer for the collection of juice. The coarse powder and juice of all was extracted with methanol and water at the ratio of 30:70. The extracts of red cabbage powder and juice were collected separately and filtered using Whatman filter paper. It was extracted with methanol and water at the ratio of 30:70 All the extracts were concentrated and the excessive solvents were evaporated under vacuum. Annona reticulata extract (ARE), Allium sativum extract (ASE), Allium. fistulosum (AFE) and Brassica oleracea extract (BOE) were named for identification.

Phytochemical screening (Qualitative)

The presence of alkaloids was determined according to the method described by Harborne38 while the method described by Odebiyi and Sofowora39 was used for flavonoids and tannins while Cardiac glycosides and saponins were determined by the methods of Sofowora40 and Wall et al.41 respectively.

Preliminary Phytochemical analysis (Quantitative)

All plant extracts were further used for chemical tests for the presence of following phytochemicals such as phenolics compounds, alkaloids, saponin, glycosides, phytosterols, tannin, flavonoids, steroids, terpenoids using the methods mentioned below:-

A). Alkaloids42

a. Mayer's test

To a few ml of filtrate, a drop or two of Mayer's reagent were reagent were added by the side of test tube. A white or creamy precipitate indicate indicated the test as positive.

b. Wagner's test

To a few ml of filtrate, few drops of wagner's reagent were added by the side of the test tube. A reddish –brown precipitate confirmed the test as positive.

B). Glycosides

a). To ml of aqueous extract of the samples, 5ml of Bendict's solution and few drop of dilute HCl were added and heated for minutes. The solution became red with precipitate which indicated the presence of glycosides.

b). Brontrager's Test43

To 2 ml of filtered hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it pink colour indicated the presence of glycosides.

C). Terpenoids44

Libermann – Burchard's test: 2ml of acetic anhydride solution was added to 1ml of petroleum ether extract of the drug in chloroform, followed by 1 ml of concentrated sulphuric acid. A violet color ring was formed indicating the presence of terpenoids.

D). Steroids45

Libermann –Burchard's test: 2 ml of acetic anhydride solution was added to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of concentrated sulphuric acid. A greenish color was developed which turned to blue.

E). Saponins46

In a test containing about 5 ml of an aqueous extract of the drug, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and left for 3 minutes. Honeycomb like froth was formed.

F). Tannins47

To 1-2 ml of plant extract, a few drops of 5% FeCL3 solution were added. A green color indicated the presence of gallotannins which brown color indicated tannins.

G). Phytosterol

a. Libermann–buchard's test
The extract (50 mg) was dissolved in 2ml acetic acid anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the side of the test tube. An array of color changes showed the presence of phytosterols.
b. The extract was treated with Salkowski's reagent
The yellowish colour with green fluorescence appearance indicated the presence of phytosterol in it.

H). Flavonoids48 & 49
SHONODA TEST: In a test tube containing 0.5 ml of alcoholic extract of the drug, 5-10 drops of dilute HCL was added followed by small pieces of magnesium. In the presence of flavonoids, a reddish pink or brown colour produced.

chemistry proceedings

MDPI

Table 1: Quantitative phytochemical screening of alcoholic and aqueous extract

Phytochemicals	Percentage composition (g/100g)							
	ARE		ASE		AFE		BOE	
	Alc.	Aq.	Alc.	Aq.	Alc.	Aq.	Alc.	Aq.
Saponins	0.73 ± 0.12	1.03 ± 0.04	0.26 ± 0.07	0.32 ± 0.02	0.26 ± 0.07	0.26 ± 0.07	0.08 ± 0.03	0.32 ± 0.04
Tannins	0.56 ± 0.23	0.13 ± 0.03	2.55 ± 0.14	2.78 ± 0.12	2.55 ± 0.14	2.55 ± 0.14	1.73 ± 0.16	2.36 ± 0.12
Cardiac glycosides	0.03 ± 0.02	0.02 ± 0.02	1.85± 0.24	1.64± 0.16	1.85± 0.24	1.85± 0.24	1.42 ± 0.17	1.90 ± 0.26
Flavonoids	3.12 ± 0.23	3.01 ± 0.18	0.08 ± 0.11	0.21 ± 0.14	0.08 ± 0.11	0.08 ± 0.11	3.03 ± 0.05	2.85 ± 0.04
Alkaloids	0.06 ± 0.01	0.46 ± 0.03	0.18 ± 0.09	0.09 ± 0.04	0.18 ± 0.09	0.18 ± 0.09	0.08 ± 0.02	0.11 ± 0.02

Values are expressed as Mean ± SEM (n = 3)

Table 2: Preliminary Phytochemical Screening of Extracts

Name of the chemical test	ARE		ASE		AFE		BOE	
	Alc.	Aq.	Alc.	Aq.	Alc.	Aq.	Alc.	Aq.
Alkaloids	-	+	+	+	+	+	+	+
Glycosides	-	-	+	+	+	+	+	+
Steroids	+	-	-	+	-	-	-	+
Flavonoids	+	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	-	+
Tannin	+	-	-	-	-	+	-	+
Terpenoids	-	-	+	+	+	+	-	+
Phytosterols	-	-	+	+	-	-	+	+

Key= + present; - absent.

Quantitative phytochemical screening of ARE indicates high concentration of flavanoids in both alcoholic as well as aquoues extracts. In ASE & AFE *extracts*, the phytochemical investigation indicates the presence of alkaloids, flavonoids, saponin, tannins and cardiac glycosides. Furthermore, the results show that the concentration of tannins (2.55 ± 0.14 g/100g) & (2.78 ± 0.12 g/100g) in the plant is the highest while flavonoids present in the plant is the lowest. In BOE, presence of Anthocynanins & polyphenols indicated by highest concentration of flavanoids (3.03 ± 0.05 g/100g) & (2.85 ± 0.04 g/100g). (Table 1)

In ARE, alcoholic extract, alkaloids are absent in other hand alkaloids are present in aqueous extract. Likewise, Glycoside, Terpenoids and phytosterols were absent in ARE alcoholic as well as aqueous extract.

In ASE & AFE, alkaloids, glycosides, steroids, flavonoids, saponins, tannin, terpenoids and phytosterols were present in aqueous extracts and steroids & tannin were absent in alcoholic extracts.

In BOE, alkaloids, glycosides, steroids, flavonoids, saponins, tannin, terpenoids and phytosterols were present in aqueous extracts and

Steroids, saponins, tannin and terpenoids found absent in alcoholic extracts.(Table 2)

Result and Discussion

In the present study, the comparison between the phytochemical property of alcoholic and aqueous extracts were estimated. The preliminary phytochemical investigation of extracts revealed the presence of various secondary metabolites such as alkaloids, glycosides, steroids, flavonoids, saponins, tannin, terpenoids and phytosterols in the different extracts.

Saponins are steroid or triterpenoid glycosides characterised by their bitter or astringent taste, foaming properties and their haemolytic effect on red blood cells. Saponins possess both beneficial (cholesterol-lowering) and deleterious (cytotoxic permeabilization of the intestine) properties and also exhibit structure dependent biological activities (Osagie and Eka, 1998). Saponins cause a reduction of blood cholesterol by preventing its reabsorption (Prohp and Onoagbe, 2012). Also, it has also been documented that saponins have antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and

viability (Roa *et al.,* 1995). Plants produce saponins to fight infections by parasites and in humans saponins help the immune system and also protect against viruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases (Prohp and Onoagbe, 2012).

Flavonoids are water soluble polyphenolic molecules and therefore belong to the polyphenol family. Together with carotenes, flavonoids are also responsible for the coloring of fruits, vegetables and Flavonoids have antioxidant activities as well as much health promoting effects viz., anti-allergic, anti-cancer, anti-oxidant, antiinflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism. Some flavonoid containing plants are diuretics (e.g. buchu), antispasmodic (e.g. liquorice) and others have antimicrobial properties (Trease and Evans, 2002). Epidemiological studies have shown that heart diseases are inversely related to flavonoid intake and that flavonoids prevent the oxidation of LDL therefore reducing the risk for the development of atherosclerosis (Prohp and Onoagbe, 2012). The presence of flavonoids in the leaves of *Cissampelos mucronata* (*Menispermaceae*) which have hypoglycaemic and anti-diabetic properties have also been documented (Tanko *et al.,* 2007). Effects of flavonoids, quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini *et al.,* (2004) as the mechanism of their hypoglycaemic activity in streptozotocin-induced diabetic rats. These are justifications for the use of the extracts of *Allium Sativum*in the treatment of diabetes mellitus. Tannins may decrease protein quality by decreasing digestibility, and palatability. Other nutritional effects which have been attributed to tannins include damage to the intestinal tract, toxicity of tannins absorbed from the gut, and interference with the absorption of iron, and a possible carcinogenic effect (Osagie and Eka, 1998). In addition, tannin has astringent properties, hastens the healing of wounds and inflamed mucous membrane. Plants with tannins are used for healing of wounds, varicose ulcers, hemorrhoids, frost- bite and burns (Igboko, 1983; Maiduyi, 1983). The presence of alkaloids in *Allium Sativum* aqueous bulb extract in this study shows the potential of the extract to have an analgesic, anti-inflammatory and adaptogenic effects, which help the host (man and animal) to develop resistance against disease and endurance against stress (Gupta, 1994). Flavonoids detected in *Allium Sativum*bulbs could be used in the treatment of various disease conditions like edema, toothache, fever, common cold, diarrhea and dental caries.

These could be possible as the root extracts contains some antibacterial activities. The flavonoids are acting on bacteria by inhibiting its protein synthesis (Hong-xi and Song, 2001).

Conclusion

From the ancient times, plants have been used for treatment of variety of disease. Thus, the present study revealed that a number of positive effects of Red cabbage such as phytochemicals were found which is beneficial for the health. The phytochemical such as alkaloids, glycosides, steroids, flavonoids, saponin, tannin, terpenoids and phytosterols were present which increases the medicinal potential of Red cabbage and thus can be used for the treatment of various diseases. Therefore, modern medicine has many side effects and it is not quite safer for human consumption. So it is better to adopt natural food which has no side effects and quite safer too for human consumption.

Acknowledgment

The authors are thankful to Dr. Mohan Kale, Principal, KGRDCP & RI, Karjat providing us lab facilities.

Reference:

1. Everett, S. L., Kowalsky, R. P., Karenchak, L. M., Landsittel, D., Day, R., Gordon, Y. J., Cornea 1995,14, 382-387.

2. Starr, C. E., Afshari, M. A., Paton, B. G., Investigative Ophthalmology & Visual Science 2000, 41, S149.

3. Cuong, V., Michael, O., Journal of Microbial Infections 2002, 4, 481-489.

4. Zaman K, Pathak K. Pharmacognostical and phytochemical studies on the leaf and stem bark of Annona Reticulata Linn. J Pharmacogn Phytochem. 2013;1:1e8.

5. Saad JM, Huri Y, Rupprecht JK, et al. Reticulatacin: a new bioactive acetogenins from Annona reticulata (Annonaceae). Tetrahedron. 1991;47:2751e2756.

6. Nirmal SA, Gaikwad SB, Dhasade VV, Dhikale RS, Kotkar PV, Dighe SS. Anthelmintic activity of Annona reticulata leaves. Res J Pharm Biol Chem Sci. 2010;1:115e118.

7. Pinto AC, Cordeiro MCR, Andrade SRM, et al. Annona Species. International Centre for Underutilized Crops. Southampton UK: University of Southampton; 2005:3e24.

8. Heinrich M, Rimpler H, Barrera NA. Indigenous phytotherapy of gastrointestinal disorders in a lowland mixe community (Oaxaca, Mexico): ethanopharmacology evaluation. J Etanopharmacol. 1992;36:63e80.

9. Prasad G. Jamkhande, Amruta S. Wattamwar, Annona reticulata Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties, Journal of Traditional and Complementary Medicine; 5, 2015, 144-152

10. Block E. Garlic and Other Alliums: The Lore and the Science. Royal Society of Chemistry. 2010.

11. Allallergy net. Retrieved April 14, Allergy Net- Allergy Advisor Find, 2010.

12. Meredith TJ and Drucker AR. Growing Garlic from True Seed. Garlic Analecta 2014.

13. Zohary D, Hopf M. Domestication of plants in the Old World, 3rd edition 2000.

14. Mikail HG. Effect of Allium sativum (Garlic) bulbs aqueous extract on T. brucei brucei infection in rabbits. M. Sc. Thesis submitted to Usman Danfodiyo University, Sokoto, Nigeria; 1995.

15. Ramasasa C. Garlic used as an effective insecticide, World Health Organization 1991. Guide lines for the assessment of herbal medicine. WHO/ TRM/ 91. Geneva: World Health Organization; 2009.

16. Vandana Singh, Ramesh Kumar, Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region, Int. J. Life. Sci. Scienti. Res., 3(6):1451-1458

17. D. Stajner, N. Milic, J. Canadanovic-Brunet, A. Kapoor, M. Stajner, B. M. Popovic, Phytother. Res. 20, 2006,581-584

18. C.Nencini, F. Cavallo, A. Capasso, G.G.Franchi, G.Giorgio, L.Miccheli, Phytother. Res. 21, 2006, 874-878

19. M. Pârvu, O., Rosea-Casian, M. Puscas, G.Groza, Contrib. Bot. 44, 125-129 (2009)

20. S. Sang, A. Lao, Y.Wang, C-K Chin, R. T. Rosen, C-T.Ho, J. Agric. Food Chem. 50. 2002, 6318-6321

21. L. Vlase, M. Parvu, E. A. Parvu, A. Toiu, Phytochemical Analysis of *Allium fistulosum* L. and *A. Ursinum* L. Digest Journal of Nanomaterials and Biostructures 8(1), 2013, 457 – 467

22. Arts IC, Hollma PC. Polyphenols and disease risk in epidemiologic studies. American Journal of Clinical Nutrition 2005; 81(1):S317-S325.

23. Lynn A, Collins A, Fuller Z, Hillman K, Rateliffe B. Cruciferous vegetables and colorectal cancer, Proc Nutr Soc. 2006; 65:135-144.

24. Fowke JH, Chung FL, Jin F, Qi D, Cai Q, Conaway C, *et al.* Urinary isothiocynate level, Brassica, and human breast cancer Cancer Res. 2003; 63:3980-3986.

25. Repetto MG and Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazil Journal Med Biol Res. 2002; 35: 523-534.

26. Jagdish Singh AK, Upadhyay A, Bahadhur B, Singh B, Singh KP, Mathura Rai AK. Antioxidant phytochemical in cabbage (*Brassica Oleracea* L. var. capitata). Scientia Horticulture 2006; 108:233-237.

27. Sterling M. Got anthocyanins. They plant pigments are more than coloring agents for fruits juices, wine and other beverages: they also contain an arry of health- promoting benefits. Nutrition science News 2000; 5:231-234.

28. Hassimotto NM, Genovese MI, Lajolo FM. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruits pulps, Journal of Agric Food Chem. 2005; 53:2928-2935.

29. Neelufar S, Alekhya T, Sudhakar K. Pharnacognostical and phytochemical evaluation of Brassica Oleracealinn var Capitataf Rubra, Journal of pharmaceutical biology. 2012; 2(2):43-46.

30. Igarashi K, Kimura Y, Takenaka. Preventive effects of dietary cabbage acylated anthocyanins on paraquat induced oxidative stress in rats. Biosci Biotecnol biochem 2000; 64:1600-1607.

31. Lee K-J, Sok DE, Kim YB, Kim MR. Protective effects of vegetables extracts on stress in brain of mice administered with NMDA Food Reseach International. Food Res Int. 2002; 35:55-63.

32. Park YJ, Jeon KH, Kim SH, Bae SJ. The effect on Antimicrobial and Cytotoxicity of *Brassica oleracea* L. Fractions, Journal of Life Sciences. 2004; 14:567-572.

33. Aml FM, Ibrahim HS, Shalaby MA. Proctective effect of broccoli and red cabbage against hepatocellular carcinoma induced by N- Nitrosodiethylamine in rats. Journal of American science 2010; 6(12):1136-1144.

34. Lin JY, Lia CY, Hwang IF. Characterisation of pigment components in red cabbage (*Brassica oleracea* L. var.) juice and their anti- inflammatory effects on LPS stimulated murine splenocytes Food Chem, 2008; 109:771-781.

35. Chaudhary A, Nagariya K, Naruka PS, Mahatma OP. Anti-inflamatory and Analgesic Activity of Whole Plant of *Brassica Oleracea* Linn Var. Capitata *F. Rubra* (Red Cabbage) in rats, Journal of Global Pharma Technology. 2010; 2(8):30-34.

36. Le Hen T, Schaldach Charlene M, Firestone Gary L, Bjeldanes, Leonard F. Plant-derived 3,3'-Diindolymethane is a Strong Androgen Antagonist in Human Prostate cancer Cells, Journal of Biological Chemical. 2003; 278(23):21136-21145

37. Kataya HA, Hamza AA. Red Cabbage (Brassica oleracea) Ameliorates Diabetic Nephropathy in Rats. Evid Based Complement Alternat Med 2008; 5:281-287.

38. Harbone, J. B., Phytochemical methods of extraction, Cox and Wymann Ltd. London, UK. 1973, 52-55, 66-70.

39. Odebiyi, O.O. and Sofowora, E.A., Phytochemical screening of Nigerian medicinal plants. Lloydia. 1978, 41(3): 234-246.

40. Sofowora, A., *Medicinal Plant and Medicine in Africa*, John Willey Spectrum, Ibadan Nigeria, 1993, Pp. 281 – 285.

41. Wall, M., Krider, M.M., Krewson, C.F., Eddy,C.R., Wiliaman, J.J., Cordell, D.S. and Gentry, H.S. Steroidal sapogeninins Xlll. Supplementary table of data for sterciJal sapogenins Vll. Agrbuftural Rsearcfi SeMce Circular. 1954; 363, 17.

42. Evans WC. Pharmacology, Harcourt Brace and Company, Asia, Singapore, 1997; 226.

43. Harbourne, JB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis, 2nd edition. Chapman, London, 1984

44. Finar IL. Stereo chemistry and chemistry of natural products, second edition, Longman, Singapur, 1986; 682

45. Kokate CK. Practical pharmacognosy, Vallabh Prakashan, New Delhi, 1994; 1: 15–30.

46. Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N and Krishnan MRV. Phytochemical investigation on a tropical plant, Pak. J. Nutri, 2009; 8: 83-85.

47. Trease GE and Evans MD. A Textbook of Pharmacognosy, Builler Tindall and Caussel, London, 1989; 13:176-180

48. Raman N. Phytochemical Techniques. New Indian Publishing Agencies, New Delhi, 2006; 19

49. Harborne JB. Phytochemical Methods, Springer (India) Pvt. Ltd, New Delhi, 2005; 17.