http://www.e-journals.net

ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2012, **9(1),** 121-130

Phytochemical and Mineral Screening of *Zanthoxylum Tetraspermum* (W & A)

K.NARAYANASAMY* and B.RAGAVAN

*Department of Biochemistry
Sree Narayana Guru College, Coimbatore - 641105, India
Department of Biochemistry, P.S.G.College of Arts and Science
Coimbatore – 641 014, Tamilnadu, India
kns_netcampus@yahoo.co.in

Received 3 June 2011; Accepted 5 August 2011

Abstract: The present study reveals the phytochemical and mineral screening of *Zanthoxylum tetraspermum* W & A., a member of the family *'Rutaceae'*, which is used for treating microbial infections, antifungal activities and tooth ache. The preliminary screening of phytochemicals, thin layer chromatographic (TLC) profile of secondary metabolites and minerals from the stem bark of *Zanthoxylum tetraspermum* have shown the presence of various important secondary metabolites such as alkaloids, flavonoids, glycosides, Phenols, sterols, saponins, tannins in higher levels in hydroethnolic and aqueous extracts and some minerals in the plant extract. The generated data has provided the basis for its therapeutic value and can be used as a therapeutant.

Keywords: Phytochemical screening, *Zanthoxylum tetraspermum*, Secondary metabolites, Mineral screening, TLC profile

Introduction

Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw materials for various scientific investigations. Many of the secondary metabolites of plants are commercially significant and find use in a number of pharmaceutical compounds[1]. Plants have almost limitless ability to synthesize aromatic substances mainly secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances act as the molecules of plant defense against predation by microbes, insects and herbivores. However, several of these molecules possess medicinal properties[2].

Herbs that were once had been the only choice for drug treatment became marginalized because of the advances in synthetic chemistry, especially in the western world. But, in due

course of time, the picture has changed and consistently being changing especially in Europe and Asia, a tremendous renaissance in the use and appreciation of herbal medicine has taken place[3,4]. A large number of minerals are present in the plants. Some of these minerals form part of body structural component and some others act as catalytic agents in many metabolic reactions. They are required in trace amounts for a wide range of functions in the cells[5]. Ayurvedic medicines are derived from the mineral, plant and animal. A number of minerals and metals are used, but they are subject to various and complex process of purification and oxidation before becoming suitable for internal usage[6].

Zanthoxylum tetraspermum is an aromatic, spiny, thicket-forming, deciduous shrub or small tree, typically attaining a height of between 2.5 and 3.5 m, some specimens up to 7.5 m. The alternate branches are armed with strong brown prickles, about 1-2 cm long, cone shaped with a broad base and found irregularly throughout the tree, but frequently at the base of young branches. It is a potent unidentified medicinal plant which is used for treating microbial infections, tumors and tooth ache[7].

In the present study, it has been focused on the preliminary screening of phytochemicals, quantitative estimation, the TLC profiling of secondary metabolites and minerals from the stem bark of *Zanthoxylum tetraspermum.*

Experimental

The whole plant material of *Zanthoxylum tetraspermum* W & A. [Syn.*Fagara tetrasperma*][8] was collected from the silent valley evergreen forest of Western Ghats, Kerala, South India. This plant belongs to the family *'Rutaceae'*.The plant was identified with the help of Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, South India.

Preliminary screening of phytochemicals

The stem barks of the plant were shade dried at room temperature for 15 days. Then the stem barks were powdered using mixer grinder and subjected to extraction.10 g of powdered bark was dissolved separately in various solvents such as petroleum ether, chloroform, Acetone, ethanol and aqueous solution for 24 hours in the order of increasing polarity of solvents. The collected 10% (w/v) extracts were used for phytochemical screening[9].

Estimation of phytochemicals

The presence of secondary metabolites from the stem bark of *Z.tetraspermum* was quantitatively estimated by adopting standard protocols. Tannins by Folin-Denis method, total phenols by Folin's cioalteau method, total carotenoids and lycopenes by Zakaria *et.al.*[10],Ascorbic acid by Roe and Keuther[11], Tocopherol by Rosenberg[12] Reduced Glutathione by Moron *et.al.*[13].

TLC profile

The thin layer chromatography (TLC) profile of various secondary metabolites was done using precoated silica gel 60 F_{254} (Merck, India) aluminium plates (20×20 cm).

Alkaloids

The powdered stem bark of *Z.tetraspermum* was wetted with a half diluted NH_4OH and lixiviated with ethyl acetate for 24 h at room temperature. The organic phase was separated from the acidified filtrate and basified with NH_4OH (pH 12.0). It was extracted with chloro form (3X) and the filtrate was condensed by evaporation and used as a sample for

chromatography. The alkaloid spots were separated using the chloroform and ethanol (15:1) solvent mixture. The colour and the R_f value of the separated alkaloid spots were recorded under visible light after spraying with Dragendorff's reagent[14].

Flavonoids

The powdered stem bark of the plant was extracted with 10.0 mL ethanol on water bath (60 ºC/5 min.). The filtrate was condensed by evaporation and added a mixture of ethyl acetate and water (1:10). Then, the content was mixed thoroughly. The ethyl acetate phase was retained and used as a sample for chromatography. The flavonoid spots were separated using the ethanol and chloroform (1:19) solvent mixture. The colour and the R_f value of the separated spots were recorded under Ultraviolet (UV_{254nm}) light[14].

Glycosides

The powdered stem bark of the plant was extracted with 80% ethanol on rotary shaker (180 thaws/min.) for 12 h. 70% Lead acetate was added to the filtrate and centrifuged at 5000 rpm /5 min. The supernatant was further centrifuged by adding 6.3% Na_2CO_3 at 5000 rpm / 10 min. The supernatant was collected and dried. It was then redissolved in chloroform and used as a sample for chromatography. The glycosides were separated using ethyl acetate-ethanol-water (40:5:5) solvent mixture. The colour and the R_f value of the separated spots were recorded under ultraviolet (UV_{254nm}) light[15].

Phenols

The powdered stem bark of the plant was lixiviated in ethanol on rotary shaker (180 thaws/min.) for 24 h. The condensed filtrate was used as a sample for chromatography. The phenols were separated using chloroform and ethanol (27:0.3) solvent mixture. The colour and the R_f values of the phenols were recorded under visible light after spraying the plates with Folin-Ciocateu's reagent and heated at 80 ºC/15 min[15].

Sterols

The powdered stem bark (1.0 g) of the plant was extracted with 10.0 mL ethanol on water bath (80 ºC/15 min.). The condensed filtrate was used as a sample for chromatography. The sterols were separated using chloroform, glacial acetic acid, ethanol and water (32:17:6:4) solvent mixture. The colour and R_f values of the separated spots were recorded under visible light after spraying the plates with a mixture of 1.0 mL concentrated sulphuric acid, 20.0 mL acetic anhydride and 50.0 mL chloroform and heated at 85-90 ºC/15 min[14].

Saponins

The powdered stem bark (1.0 g) of the plant was extracted with 10.0 mL 70% ethanol by refluxing for 10 min. The condensed filtrate was enriched with saturated *n*-butanol and thoroughly mixed. The butanol phase was retained, condensed and used as a sample for chromatography. The saponins were separated using chloroform, glacial acetic acid, ethanol and water (32:17:6:4) solvent mixture. The colour and R_f values of the spots were recorded by exposing the chromatogram to the iodine vapours[14].

Minerals screening

Atomic absorption spectrophotometry has become a standard method for the analysis of mineral content of plant sources[5]. Hence, the stem barks of the plant were powdered using mixer grinder and 1.0 g of the powdered bark was taken in a silica crucible and heated in a muffle furnace till there was no evolution of smoke. Then, the crucible was cooled at room

temperature and the ash formed was moistened with concentrated sulphuric acid. It was heated on a heating mantle and the ash obtained was dissolved in 5% hydrochloric acid solution. This solution was used for determination of various mineral elements such as calcium, iron, sodium, potassium, phosphorous, zinc and magnesium by using an atomic absorption spectrophotometer and total nitrogen content by Kjeldahl method[9].

Results and Discussion

Phytochemicals screening

The medicinal plants are having various secondary metabolites such as alkaloids, Flavonoids, glycosides, phenols, tannins, saponins, sterols *etc*. The preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of the pharmacologically important active chemical components[16]. The stem bark extracts of the *Z.tetraspermum* have revealed the presence of alkaloids, flavonoids, glycosides, lignins, phenols, tannins, sterols, thiols, saponins, fats and oils *etc*. The results of the phytochemical screening are shown in the Table 1. Among the various extracts, ethanolic and aqueous extracts were known to be rich in the phytochemicals.

Table 1. Phytochemical screening of *Zanthoxylum tetraspermum*

S.No.	Secondary Metabolite	Name of the test	Plant stem bark 10% extract				
		Catechin test.	A	B	C	D	E
		Gelatin test.	+	+	++	++	+++
1.	Tannins	Chlorogenic acid test.	-	-	-	-	-
		Lead acetate test.	-	+	-	++	-
		Ferric chloride test.	-	-	++	+	+++
		Shinoda test.	+	+	++	+++	+
2.	Flavonoids	NaOH test.	-	-	++	+++	+++
		Zinc hydrochloride test.	-	-	++	+++	+++
		Dragendorff's test.	-	-	++	+++	+++
		Mayer's test.	-	-	++	++	++
		Wagner's test.	-	-	++	++	++
3.	Alkaloids	Hager's test.	+	+	++	++	+++
		Tannic acid test.	-	-	++	++	++
		Picrolonic acid test.	-	-	++	++	+++
		Phosphotungstic acid test.	-	-	++	++	+++
		Borntrager's test.	-	+	++	++	+++
		Raymond's test.	-	-	++	++	+
4.	Glycosides	Legal's test.	-	-	++	+	+
		Kedde's test.	-	-	++	+	-
		Kellar-kiliani test.	-	-	-	-	-
		Phloroglucinol test.	-	-	-	-	-
5.	Lignin	Saffranine test.	-	-	+++	++	+
		Thionine test.	-	-	+++	++	+
6.	Fat & Oils	Sodium bisulphate test.	-	-	+++	++	+
		Copper sulphate test.	-	-	++	++	++

Contd…

7.	Thiols	Thiols test.	++	++	-	-	-
8.	Saponins	Foam test.	+++	+++	++	+	+
		Haemolysis test.	-	-	+	++	+++
		Sulfur test.	-	-	+	++	+++
9.	Sterols	Libermann-Burchard test.	++	++	++	-	-
		Salkowski test.	++	++	++	-	-
10.	Cellulose	Iodine test.	++	++	++	+	++
11.	Inulin	Inulin test.	+	+	+	++	+
12.	Carbo Hydrates	Molisch's test.	+	++	++	++	++
		Fehling's test.	++	++	++	++	++
		Benedict's test.	++	+	++	++	++
13.	Proteins	Millon's test.	+	++	++	++	+
		Biuret's test.	-	-	++	++	+
		Xanthoproteic test.	-	-	++	++	++

Note: '+++' ⟶ High; '++' ⟶ Moderate; '+' ⟶ Low; '-' ⟶ Absent. And A= Petroleum ether extract, B= Chloroform extract, C=Acetone extract, D=ethanol extract, E=Aqueous extract

Phytochemical estimation

The phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plant has shown that the stem bark was rich in secondary metabolites. They were known to show medicinal activity as well as exhibiting physiological activity[17]. The data of quantitative estimation of secondary metabolites from the stem bark of *Z.tetraspermum* is shown in the Table 2. Among the seven groups of phytochemicals estimated from the stem bark, phenols were found to be the most abundant one, followed by tannins, vitamin E, reduced glutathione and ascorbic acid (vit.C).Whereas, the carotenoids and lycopenes were reported in lower concentrations.

Table 2. Estimation of phytochemicals from *Zanthoxylum tetraspermum*

S.No.	Phytochemical	Percent of phytochemical / dry weight (g %)
1.	Tannins.	0.80
2.	Phenols.	4.44
3.	Carotenoids.	0.12
4.	Lycopenes.	0.06
5.	Ascorbic acid. (Vitamin C)	0.12
6.	Tocopherol. (Vitamin E)	0.64
7.	Reduced Glutathione.	0.15

TLC profiling

The data of TLC profile of secondary metabolites from the stem bark of *Z.tetraspermum* is tabulated in the Table 3. Among the six different solvent extracts of the plant, ethanol and aqueous extracts were reported intense spots.

Alkaloids are one of the important classes of secondary metabolites which are heterocyclic indole compounds which have shown to contain pharmacological properties such as hypotensive activity[18], anticonvulsant activity[19], antiprotozoal, antimicrobial activites[20] and antimalarial activity[21]. They are used as analgesics, stimulants, anaesthetic, hallucinogens and antibacterial agents[22].

Table 3. TLC profile of phytochemicals of *Zanthoxylum tetraspermum*

S.No.	Phyto-Chemical	Stem Bark Extracts											
		A		B		C		D		E		F	
		Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value	Colour of the spot	R_f value
1.	Alkaloids	No spot	-	Intense purple	0.79	Light purple	0.83	Purple	0.69 0.90	Intense purple	0.90	Purple	0.86
2.	Flavonoids	No spot	-	Green	0.44 0.58 0.80	Green	0.33 0.96	Green	0.94	Green	0.91	Green	0.91
3.	Glycosides	No spot	-	No spot	-	No spot	-	Greenish purple	0.27	Greenish yellow	0.26 0.88	No spot	-
4.	Phenols	No spot	-	No spot	-	Light Blue	0.77	No spot	-	Intense Blue	0.78	Intense Blue	0.19
5.	Sterols	Yellowish purple	0.69 0.94	Intense yellowish purple	0.92	Intense yellowish purple	0.94	yellowish purple	0.98	yellowish purple	0.55 0.94	Yellowish purple	0.58 0.94
6.	Saponins	No spot	-	No spot	-	Yellow	0.71 0.98	Yellow	0.68 0.98	Intense yellow	0.76 0.95	Intense yellowish orange	0.22

A= Petroleum ether extract, B= Chloroform extract, C=Acetone extract, D=Ethanol extract, E= Aqueous extract, F= 50% Ethanol extract

$$\text{Relative front } (R_f) \text{ value} = \frac{\text{Distance moved by the substance}}{\text{Distance moved by the solvent}}$$

Moreover, two benzophenanthrene alkaloids such as 8-aetonyldihydronitidine and 8-aetonyldihydroavicine have been isolated from the stem bark of the *Z.tetraspermum* and they have shown significant antibacterial and anti fungal activity[23]. Ethanol extract has shown two different spots with R_f values 0.69, 0.90.Aqueous extract (R_f value 0.90) and ethanol extract have reported clear spots. No spot was reported by petroleum ether extract.

Flavonoids are the phenolic substances which are the largest group of phenols. They generally occur as a C6-C3 unit linked to an aromatic ring[16]. They are other plant constituents with antibacterial and antifungal properties. They are used in treating stomach ulcer and inhibit HIV -1 integrase and HIV -1 protease enzymes which are responsible for the HIV replication[24]. Three different flavonoid spots were reported from chloroform extract with R_f values 0.44, 0.58, 0.80. The acetone extract of the plant has reported two spots with R_f values 0.33 and 0.96.The ethanol, 50% ethanol and aqueous extracts of the plant have reported one spot each with R_f values 0.94, 0.91 and 0.91 respectively. No spot was observed in the petroleum ether extract.

The data of glycosides of *Z.tetraspermum* by TLC has shown colour spots both on ethanol (R_f value 0.27) and aqueous extracts (R_f values 0.26, 0.88) only. No other extracts of the plant has reported the spots. The antimicrobial activities of the medicinal plants may be related to the presence of glycosides and saponins[25]. The glycosides are reported to possess strong anti-bacterial activities and exist as antibiotics[26].

Plants have limitless ability to synthesize phenols or their derivatives. The presence of phenols in all types of tissues is a characteristic feature of plants[14]. The data of phenols of *Z.tetraspermum* has reported one blue coloured spot each on acetone, aqueous and 50% ethanolic extracts with R_f values 0.77, 0.78 and 0.19 respectively. No coloured spot was observed on the other extracts.

Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones. They are given to breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones[27]. The data of sterols of stem bark of the plant has revealed the presence of two spots each on petroleum ether, aqueous and 50% ethanolic extracts of the plant. Whereas, the chloroform, acetone and ethanol extracts have reported only one spot each with R_f values 0.92, 0.94 and 0.98 respectively.

Saponins are glycosides of both triterpenes and sterols generally possessing five sugar units and gluconic unit as a component. The occurrence of saponins has been reported in over seventy families of higher plants[28]. They are used to reduce body cholesterol by preventing its re-absorption. They also find applications in foaming fire extinguishers, emulsifiers and insecticides[22]. The data of saponins of *Z.tetraspermum* has reported two spots each on acetone, ethanol and aqueous extracts. One spot has been observed in 50% ethanolic extract of the plant (R_f value 0.22).Whereas, petroleum ether and chloroform extracts of the plant have not reported any spots.

Minerals screening

Minerals are essential for good health. The body utilizes over 80 minerals for maximum functions. Minerals function as co-factors, enabling the body to perform its functions including energy production, growth and healing. Trace minerals have a therapeutic effect on the quality of life of cancer patients. The main therapeutic aim in malignancies is to improve the immune system. Minerals are essential to the functions of the immune system[29]. Mineral delivery into the cell interior is important because many of the cell's cytoplasmic and mitochondrial enzymes require minerals in order to be activated[30].

Ayurveda tells us that minerals increase fire and that fire represents the principle of transformation. In the body, transformation means the metabolism or digestion and assimilation of our food. In modern terms, minerals are needed to support all enzyme activities which govern metabolism. Therefore, a mineral deficiency or imbalance can disrupt enzyme activity and metabolism[6].

Ayurvedic medicinal preparations are complex mixtures including plant derived products, minerals and metals.Sankha, praval (muga). Sipi, iron, gold, moti, tama, zinc, mercury *etc.* are used to prepare ayurvedic medicine from minerals. Some examples are sankha bhasma, praval bhasma, swarna bhasma, rhumayog gold, tribhuvan kirti ras *etc*[6].

The various minerals such as calcium, iron, sodium, potassium, phosphorous, zinc, magnesium and nitrogen *etc*, were analyzed in the plant ash sample. The ash content of stem bark of the *Z.tetraspermum* has revealed the presence of all these mineral elements in adequate quantities. The results of the mineral screening are represented in the Table 4. Among the minerals found in the plant ash, calcium, sodium and total nitrogen content were reported in higher quantities.

Table 4. Minerals screening of *Zanthoxylum tetraspermum*

S.No.	Minerals	mg/100 g of the Plant sample, mg %
1.	Calcium.	941.66
2.	Iron.	44.83
3.	Sodium.	507.26
4.	Potassium.	4.17
5.	Phosphorous.	30.00
6.	Zinc.	9.46
7.	Magnesium.	42.50
8.	Total Nitrogen	938.78

Calcium aids in stopping varieties of cancer by improving DNA's capability to recover from deterioration. Consuming calcium health supplements could decrease women's opportunity of breast cancer up to 40%[27]. Nutritional supplement usually bound to calcium helps act as a cell membrane sealant reducing cell entry of toxins and viruses and it helps maintain and improve the electrical potential of cell membranes particularly in cells involved in inflammatory processes[31].

Iron strengthens immune function and its deficiency may also increase susceptibility to infection. It plays a role as part of an enzyme necessary for DNA synthesis and is needed for reproduction, growth and healthy immune system functions. Nitrogen is another important element which plays a vital role in digestion and growth[29].

Phosphorous is present in almost all plants. It is one of the three main elements that make plant life possible. It is a part of the body's energy storage system and found in substantial amounts in the nervous system. It forms a part of every cell membrane and is vital for growth and repair[29].

Zinc is an extremely vital mineral for many functions of the body. It is integral to the synthesis of RNA, DNA, aiding enzymes in digestion, energy metabolism and wound healing. It is a critical nutrient of immunity as it is involved in cell mediated and antibody-mediated immunity. It also possesses direct antiviral and antioxidant activity[29]. Dietary correction of essential intracellular mineral deficiencies like zinc and other trace elements is also critically important. An example would be the very similar cancer diets promoted by Dr. Hans Nieper[31].

Intracellular sodium-potassium ratio with excessive intracellular sodium and low intracellular potassium could affect the transmembrane potential of malignant cells[32]. Magnesium is a key substance in the proper functioning of nerves and muscles. It is a catalyst in building proteins, producing energy, involved with muscle contraction, transmission of nerve impulses and supports the healthy immune system. Intracellular concentration of magnesium will result in cell water becoming more structured and will cause the cell to release unstructured cell water and sodium[33,34].

The potassium and zinc were found in lower quantities in the plant ash. The other three elements namely phosphorous, magnesium and iron were present in moderate quantities. Similar kind of estimation of trace elements content of *carthamus oxyacantha, Eruca sativa* and *plantago ovata* have been done by Ellahi *et.al*[35]. Several other medicinal plants like *Achyranthes aspera*, *Alternanthera pungens*, *Cannabis sativa*, *Justicia adhatoda*, *Parthenium hysterophorus*, *Withania somnifera*, have been studied first time for their trace and major elemental composition by atomic absorption spectrophotometer[36].

Conclusion

Therefore, the data generated from these experiments have provided the chemical basis for using this plant as a therapeutic agent for treating various ailments. However, there is a need to further carry out advanced spectroscopic studies in order to elucidate the structure of these compounds. Moreover, this generated data maybe useful and handy in probing the biochemical characteristics of this plant in the future.

Acknowledgment

The authors are thankful to the Principal of PSG College of Arts and Science, Coimbatore, Dr.Asha Balagangadharan, Principal, Sree Narayana Guru College, Coimbatore, Mr.V.P.Prabhakaran, secretary, Sree Narayana Guru Educational Trust, Coimbatore for giving permission to carry out this work.

References

1. Joy P P and Thomas J, Samuel Mathew and Baby P Skaria, Medicinal Plants; Kerala Agricultural University, 1998.
2. Cowan M M, *Clin Microbiol Rev.,* 1999, **12,** 564-582.
3. Farnsworth N R, Akerele O, Bingel A S, Soejarto D D and Guo Z, Medicinal plants in therapy, Bull WHO, 1985, **63**, 965-981.
4. Winslow L C and Kroll D J, Herbs as Medicine, *Arch Inter Med*., 1998, **158**, 2192-2199.
5. Gopalan C, Rama Sastri B V and Balasubramanian S C, Nutritive value of Indian foods, NIN, ICMR, Hyderabad, India, 2004.
6. Sukh Dev, Ancient-Modern Concordance in Ayurvedic Plants: Some Examples, Environmental Health Perspectives, University of Delhi, B.R.A. Centre for Biomedical Research, Delhi, India, 1999, **107(10),** 783-789.
7. Nayar M. P, Meaning of Indian flowering plant names, 1985, Publisher, Bishen Singh Mahendra Pal Singh, Digitized, Feb 2009.
8. The Indian Forester, 1958, Vol. 84, Publisher, R P Sharma, digitized, Nov 2007.
9. Harborne J B, Phytochemical Methods: A Guide to Modern Techniques of Plant analysis; 2nd Ed., Chapman and Hall, New York, 1998, 1-302.
10. Rosenberg H R, Chemistry and Physiology of the Vitamins, 1992, Inter Science Publisher Inc.

11. Moron M S, J N De Pierre and Manervik V, *Biochem Biophys Acta*, 1979, 582, 67-68.
12. Wagner R and Bladt S, Plant Drug analysis, A Thin layer Chromatography Atlas, 2nd Ed., 1996, Springer, Berlin.
13. Harborne J B, Phytochemical methods; 3rd Ed., Chapman and Hall, Madras, 1998.
14. Mallikharjuna P B, Rajanna L N, Seetharam Y N and Sharanabasappa G K, *E-J Chem.,* 2007, **4**, 510-518.
15. Sofowara A, Medicinal plants and Traditional medicine in Africa; Spectrum Books Ltd, Ibadan, Nigeria, 1993, 289-300.
16. Ali M M and Ghatak B J, *Ind J Exp Biol.,* 1975, **13(2),** 163-167.
17. Singh H and Kapoor V K, *Planta Med.,* 1980, **38(2)**, 133-137.
18. Quetin-Lacqlercq J, Favel A, Balansard G, Regli P and Angenot L, *Planta Med.,* 1995; **61,** 475 - 480.
19. Frederich M, Jacquier M J, Thepenier P, De Mol P, Tits M, Philippe G, Delaude C, Angenot L and Zaches Hanrot M, *J Nat Prod.,* 2002; **65,** 1381.
20. Dangoggo S M, Faruq U Z and Hassan L G, *J Veterinary Sci.,* 2002; **4(2),** 8 - 11.
21. Ajith P K, Nissanka *et al,* Antimicrobial alkaloids from *Zanthoxylum tetraspermum* and *caudatum*, *Phytochemist.* 2001; **56(8),** 857-861.
22. Gafar M K, Hassan L G, Dangoggo S M and Itodo A U, *J Chem Pharm Res*., 2010, **2(5),** 277-285
23. Dangoggo S M, Faruq U Z and Manga S B, *J Phys Math Sci*., 2001, **1(1),** 29-33.
24. Dangoggo S M, Faruq U Z and Manga S B, *Nigerian J Phy Math Sci.,* 2001, **1(1),** 29-33.
25. Okwu D E, *Global J Pure Appl Sci.,* 2001, **7(3),** 455- 459.
26. Hostettmann K and Marston A, Saponins, Cambridge University Press, UK, 1995.
27. Helen A, Norman, Ritva R, Butrum, Elaine F, David Heber, Daniel Nixon, Mary F P, Richard R, Artemis Simopoulos, Michael J. Wargovich, Elizabeth K, Weisburger and Steven H Z, *Nutr.,* 2003, **133,** 3794S - 3799S.
28. Karppanen H, Karppanen P, Mervaala E, *J Human Hypertension.,* 2005, **19**, S10-S19.
29. Nieper H A, The colamine phosphate salts as membrane integrity factor; Raum and Zeit Aug, 1988, **35**, 4-9.
30. Cone C D, *Ann NY Acad Sci.,* 1975, 238, 420-35.
31. Cope F W, *Physiol Chem Phys.,* 1978, **10(5)**, 465 - 468.
32. Ling G N, *Agressologie.,* 1983, **24(7),** 293- 302.
33. Ellahi Bukhsh, Salman Akbar Malik and Sheikh Saeed Ahmad, *Pak J Bot.,* 2007, **39(4),** 1181-1187.
34. Shazia Jabeen, Muhammad T S, Sardar Khan and Muhammad Qasim Hayat, *J Med Plants Res.,* 2010, **4(7)**, 559 - 566.
35. Zakaria H, Simpson K, Brown P R and Krotulovic A, *J Chromatography*, 1979, **176**, 109-117.
36 Roe J H and Keuther C A, *J Biol Chem*.,1953, **147,** 399-407.