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Chemical profile studies on the secondary metabolites of medicinally important plant *Zanthoxylum rhetsa* (Roxb.) DC using HPTLC

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ABSTRACT

Objective: To establish the chemical fingerprint of various secondary metabolites of *Zanthoxylum rhetsa* (Roxb.) DC, a medicinally important plant. **Methods:** Preliminary phytochemical screening for various secondary metabolites was carried out. HPTLC profiles of various individual secondary metabolites were done and profiles were developed for authentication. **Result:** The ethanolic extract of the fruit showed the presence of 8 Glycosides, 10 Flavonoids, 6 Essential Oils, 5 Anthraquinones, 9 bitter principles, 7 Coumarins and 8 Terpenoids. **Conclusions:** The development of such fingerprint for the fruits of *Zanthoxylum rhetsa* (Roxb.) DC is useful in differentiating the species from the adulterant and also act as biomarker for this plant in the Pharmaceutical industry.

1. Introduction

Natural products from botanicals can be regarded as biologically validated structural entities, since they were synthesized by proteins and therefore are highly likely to bind to similar motifs again [1]. Plants may be regarded as libraries of small molecule secondary metabolite organic compounds with considerable structural diversity, which would otherwise probably be unavailable in a synthetic chemical laboratory [2-5]. Plants have developed chemical defenses over millions of years against environmental threats such as UV radiation, reactive oxygen species and microbial attacks. Therefore phytochemicals are less toxic and biologically active [6].

The current scenario exhibits the demand for plant drugs throughout the world. The quality, efficacy and safety of herbal drugs can be ensured by finding reliable characteristics, one of which would be determination of all the phytochemical of the extract. This would ensure the reliability and repeatability of pharmacological and clinical research to understand their bio-activity and to enhance the product quality control. Development of chemical fingerprints using HPTLC is an effective tool for linking the chemical constituents' profile of the plant with botanical identity for estimation of chemical and bio chemical markers. It is efficient and economic for the analysis of broad number of compounds [7]. It has the potential to determine authenticity and reliability of chemical constituent of herbal drug and formulation.

Zanthoxylum rhetsa (Roxb.) DC belonging to family Rutaceae is known as tirphal and is distributed in Konkan, Deccan, Mysore, Malabar, Annamalais and Travancore at low elevation, Orissa, Chittagong , Pegu. Also found in Assam and Meghalaya and in Eastern and Western Ghats of peninsular India [8]. The fruits exhibit the medicinal properties such as stimulants, astringent, aromatic and digestive [8]. Essential Oils from fruit shows significant local anesthetic activity [9,10]. Phytochemical studies have been carried out by several workers on *Zanthoxylum rhetsa* (Roxb.) DC, revealing the presence of various secondary metabolites. The plant is traditionally used as antidiabetic, antispasmodic, diuretic and anti-inflammatory activities in other regions of India. The plant also bears significant antinociceptive and antidiarrheal activities [11,12]. Chemically the plant contains a terpenoid, xanthyletin and sesamin, alkaloids and flavonoids and an essential oil, sabinene as its key constituents [13,14,15].

But study on development of individual secondary metabolites of fruits of *Zanthoxylum rhetsa* (Roxb.) DC by HPTLC has not yet been reported. With this background an attempt has been made to establish a profile of the various

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secondary metabolites of fruits of *Zanthoxylum rhetsa* (Roxb.) DC.

Material and Methods:

Collection & Identification:

The fruits of the plant *Zanthoxylum rhetsa* (Roxb.) DC were collected in the month of May – June 2010 from Sindhudurg district, Maharashtra, India. They were identified and authenticated at Blater's Herbarium, St Xavier's College, Mumbai. (Accession numbers 9046/2010). The fruits were air dried pulverized and used for analysis.

Chemicals:

All the chemicals, including solvents were of analytical grade. The HPTLC plates Si60F254 (10X 10) Meck of 0.2mm thickness were used as stationary phase.

Extraction:

The powdered samples were extracted with ethanol by continuous shaking for 6 hours. The extract was then allowed to stand for 18 hours. The filtered extract was then concentrated and redissolved in ethanol. The samples were used for developing HPTLC fingerprint profile of various secondary metabolites.

Preliminary Phytochemical Analysis

Qualitative phytochemical analysis of crude powder was determined as follows:

Tannins (200mg powder in 10ml distill water, filter).A 2 ml filtrate + 2ml FeCl3 blue black precipitate indicated the presence of tannins. Alkaloids (200mg in 10ml methanol, filtered) A 2ml filtrate + 1% HCl + steam. 1ml filterate + 6 drops of Mayers/ Wagners reagent/Dragendroff's reagent. Creamish precipitate/Brownish red precipitate/ orange precipitate indicated presence of respective alkaloids. Saponins (frothing test: 0.5ml filterate+5ml distilled water), frothing persistent meant saponins present. Cardiac glycosides (Keller Killani test), green blue colour indicated the presence of cardiac glycosides [16], Anthraquinones [17], Steroids (Libermann–Burchard reaction) blue green ring indicated presence of terpenoids. Flavonoids (200mg powder in 10ml ethanol, filtered). A 2 ml filtrate + conc. HCl + magnesium ribbon. Pink tomato red colour indicated the presence of flavonoids [18].

HPTLC Fingerprinting:

The HPTLC analyses were performed on aluminum plates pre–coated with silica gel 60F254 (Merk, Germany). 10 μl of each extract were applied on the plate of 10 X 10 cm as bands of 10 mm width of each with the help of CAMAG linomat IV sample applicator. The plates were developed in a CAMAG twin– trough chamber previously equilibrated with a mobile phase for 20 minutes. Different solvent systems were used to develop HPTLC fingerprint profile for different secondary metabolite groups separately [18,19,20]. Each plate were developed up to 8 cm, air dried and scanned at wavelength of 254 & 366 nm using CAMAG TLC Scanner 3. The chromatograms were recorded. Then the plates were derivatized with respective chemical reagents and heated at 105 0C on hot plate till the development of colour of bands and observed under white light. The colour of recorded bands and Rf values were recorded.

Results:

The current study was oriented towards the screening of *Zanthoxylum rhetsa* (Roxb.) DC for secondary metabolites and development of fingerprints using HPTLC technique. Preliminary Phytochemical analysis revealed the presence of alkaloids, steroids, flavonoids, Anthraquinones, glycosides, Terpenoids, and essential oils (Table 1). HPTLC screening showed the presence of different types of bitter principles, coumarins and phenol carboxylic acids in addition to the compounds detected by preliminary screening.

Table No. 1:

Preliminary screening of secondary metabolites from ethanolic extract of fruits of *Zanthoxylum rhetsa* Roxb (DC)

Sr. No.	Secondary metabolites	Test	Inference
1.	Alkaloids	Dragendorff' test	+
2.	Glycosides	Molisch test	+
3.	Saponins	Frothing test	+
4.	Steroids	Shinoda test	+
5.	Anthraquinones	Mg– acetate test	+
6.	Terpenoids	Salkowski test	+
7.	Tannins	FeCl3 test	–
8.	Essential Oils	Odour	+

Cardiac Glycosides

The HPTLC chromatogram for cardiac glycosides was best observed at 254nm & 366 nm before derivatization. There is no need for derivatization since compounds are seen best separated before derivatization. 8 bands of Cardiac glycosides are seen to be separated before derivatization at 366 nm. The compounds separated were seen at Rf = 0.13, 0.29, 0.39, 0.45, 0.50, 0.64, 0.70, 0.77. Best Solvent system to observe the above separation is: Ethyl Acetate: Methanol: Water (10: 1.4:1)

Flavonoids

The HPTLC chromatogram can be best observed under fluorescence 254nm &366 nm before and after derivatization. 10 bands of flavonoids are seen to be separated before

derivatization at 366 nm. The major compounds separated were seen at Rf = 0.01, 0.16, 0.19, 0.28, 0.31, 0.37, 0.50, 0.60, 0.77& 0.84. Best Solvent system to observe the above separation is: Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water (10:0.5:0.5:1.3)

Essential oils

The HPTLC chromatogram for Essential oils can be observed at 254 nm and 366nm before derivatization. 6 bands of Essential oils are seen to be separated before derivatization at 366 nm. The major compounds separated were seen at Rf = 0.27, 0.39, 0.54, 0.67, 0.77, 0.84. However there is still need for improvements and modification in the process of extraction in order to optimize the concentration of separation of bands. Solvent system: Toluene: Ethyl Acetate (9.3:0.7)

Anthraquinones

The HPTLC chromatogram for anthraquinones was best observed at 366 nm before and after derivatization. 5 bands of Anthraquinones were seen to be separated. The major compounds separated were seen at Rf = 0.00, 0.05, 0.17, 0.61& 0.78. Best Solvent system: Ethyl Acetate: Methanol: Water (7.7: 1.5:0.8)

Bitter principle

The HPTLC chromatogram can be observed \at wavelength 254 nm and 366 nm before derivatization. 9 major compounds were separated and seen at Rf = 0.00, 0.07, 0.12, 0.32, 0.50, 0.55, 0.66. 0.78 and 0.87.

Coumarins

Coumarins can be observed at wavelength 254 nm and 366 nm before derivatization. 7 bands of Coumarins are seen to be separated before derivatization at 366 nm The major compounds separated were seen at Rf = 0.13, 0.19, 0.30, 0.50, 0.60, 0.67 and 0.74.

Terpenoids

Terpenoids can be best observed at 366 nm before derivatization. There is need for derivatization as they are seen to be best separated before derivatization. 8 bands of Terpenoids are seen to be separated before derivatization at 366 nm. The major compounds separated were seen at Rf = 0.06, 0.11, 0.23, 0.26, 0.57, 0.69, 0.83, and 0.87. However there is still need for improvements and modification in the process of extraction in order to optimize the concentration of separation of bands. Solvent system: n-Hexane: Ethyl Acetate (1:1)

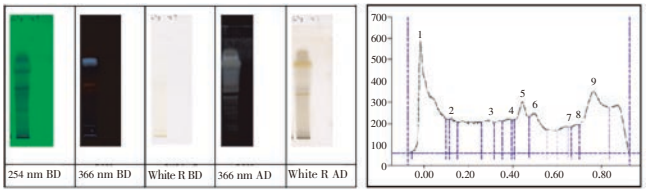

Plate No. 1: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Glycosides

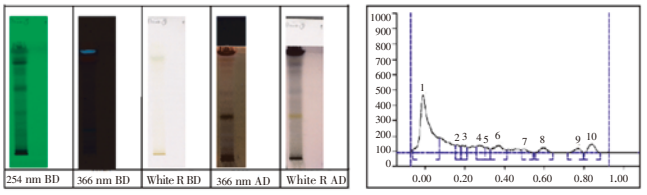

Plate No. 2: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Flavanoids

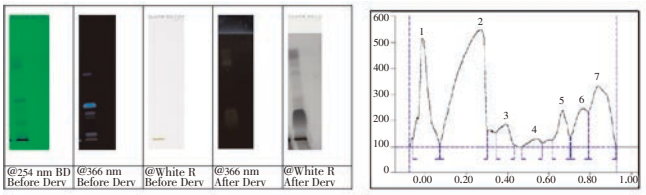

Plate No. 3: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Essential Oils

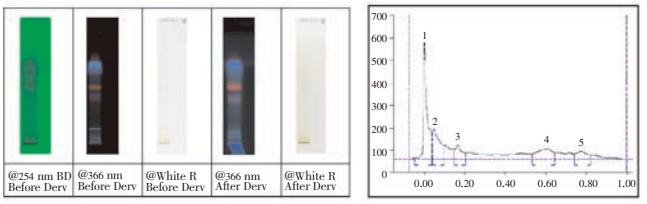

Plate No. 4: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Anthraquinones

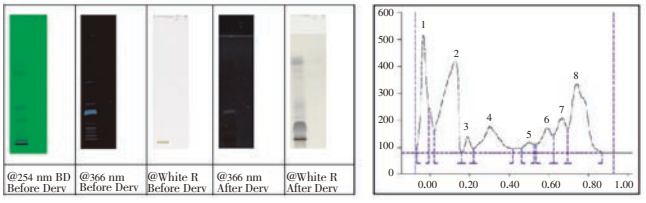

Plate No. 5: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Coumarins

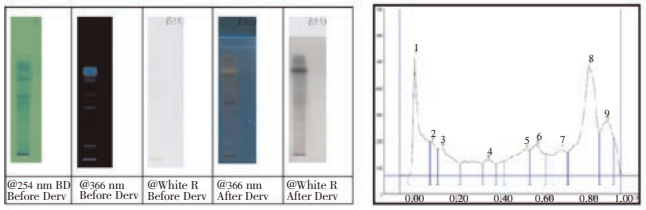

Plate No. 6: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Bitter Principles

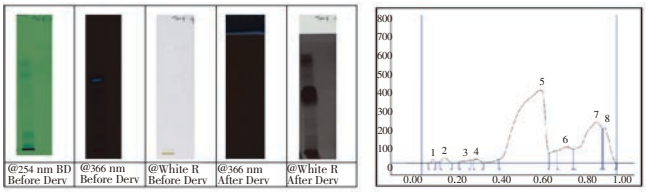

Plate No. 7: HPTLC fingerprint of *Zanthoxylum rhetsa* Roxb (DC) of Terpenoids

Discussion:

Nature produces a tremendous array of secondary metabolites or natural products with most diversity seen in microorganisms and plants [21, 22]. Natural products are the main sources of bioactive molecules and have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases [23]. Among the bioactive secondary metabolites present in medicinal plants alkaloids, biflavanoids ,isoprenoids are of high interest. Discoveries of anticancer compounds have encouraged the total synthesis of these compounds [24, 25, 26]. These compounds have important ecological functions providing as defense mechanisms and also serving as attractants for pollinators and seed dispersing agents. They also contribute to allelopathy. Humans exploit natural products as a source of drugs, flavouring agents, fragrances and for a wide range of other applications. [6].

A vast array of secondary metabolites was found to be present in the plant under study. The ethanolic extract of the fruit showed the presence of 8 Glycosides, 10 Flavonoids, 6 Essential Oils, 5 Anthraquinones, 9 bitter principles, 7 Coumarins and 8 Terpenoids.

Cardiac- glycoside based drugs increasingly being used for cancer treatments Cardiac glycosides comprise a large family of naturally derived compounds. They show considerable structural diversity, but all members of this family share a common structural motif.

A good separation of Flavanoids has been observed. The anti oxidant activity of phenolics and polyphenolics is often regarded to be the basis of health promoting activity. Flavanoids are reported to have anti oxidant anticancer anti allergic, antiinflamatory, anti carcinogenic and gastroprotective properties [27,28,29,30].

5 bands of Anthraquinones were seen to be separated. Anthraquinones, as the largest group of naturally occurring quinones, are of importance both in industry [31] and in medicine [32]. Anthraquinones derivatives (AQ) have aroused special interest since they have demonstrated potential therapeutic uses as antibacterial, antiviral, antifungal agents and other biological activities [33-37]. Plants containing flavonoids and anthraquinones (such as quercetin and emodin) are good antibacterial agents against many human pathogenic bacteria such as Escherichia coli, Streptococcus sp, Staplylococcus aureus and Pseudomonas aeruginosa [38-41].

A good separation of essential oils has been observed. 6 bands of essential oils were seen to be separated. Essential oils have been traditionally used for treatment of infections and diseases all over the world for centuries [42]. Essential oils from different plant species possess ovicidal, larvicidal, and repellent properties against various insect species and are regarded as environmentally compatible pesticides [43-44]. Volatile compounds from plants,especially essential oils have been demonstrated to possess potent antifungal, antibacterial, insecticidal and nematocidal activity [45-51].

A good separation of Coumarins has also been observed. 7 Coumarins were seen to be separated. Coumarins have multiple biological activities including disease prevention, growth modulation and anti-oxidant properties. These compounds are known to exert anti-tumour effects and can cause significant changes in the regulation of immune responses, cell growth and differentiation [52].

The coumarins are of great interest due to their biological properties. In particular, their physiological, bacteriostatic and anti-tumour activity makes these compounds attractive for further backbone derivatisation and screening as novel therapeutic agents, [53] and it was shown that coumarin and its metabolite 7-hydroxycoumarin have antitumour activity against several human tumour cell lines. Both coumarin and coumarin derivatives have shown promise as potential inhibitors of cellular proliferation in various carcinoma cell lines [54-56]. In addition it has been shown that 4-hydroxycoumarin and 7-hydroxycoumarin inhibited cell proliferation in a gastric carcinoma cell line [33].Coumarins can be used not only to treat cancer but to treat the side effects caused by radiotherapy.

Pharmaceutical and food industries have exploited terpenes for their potentials and effectiveness as medicines and flavor enhancers [57]. Terpenes have also shown antimicrobial activities This is important due to the increase in antibiotic resistant bacteria, which is occurring globally and at an alarming rate [58,59,60].

Conclusion:

Standardisation of plant materials is the need of the day. An HPTLC fingerprint is suitable for rapid and simple authentication. The HPTLC fingerprint developed may serve as a supplement chromatographic data and the information thus generated may be explored further as a tool for standardization. HPTLC analysis revealed a better separation of individual secondary metabolites. The plant can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. These plant derived bioactive compounds in addition of being developed directly as drugs can also served as prototype drug molecules known as "Lead

Compounds" and as pharmacological probes to help better understand biochemical and physiological mechanisms [6]. Bioactivity guided fractionation can lead to the isolation of active principle of this plant and some of the chemical entities with acceptable pharmaceutical qualities can be developed as drugs in their original form directly. In addition to their medicinal use some secondary metabolites from these plants can also serve as powerful "pharmacological tool" to help explain the mechanism underlying human diseases [61,62].

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Schwarz E, Metzler J, Diedrich JP, Freudenstein J, Bode C, Bode JC. Oral administration of freshly expressed juice of Echinacea purpurea herbs fail to stimulate non specific immune response in healthy young men: results of a double blind, placebo-controlled cross over study. *J Immnunother.* 2002; **25**:413-20

[2] Bindseil KU, Jakupovic J, Wolf D, Lavayre J, Leboul J, van der PYL D; 2001; Pure compound library a new perspective for natural product based drug discovery. *Drug Discov Today*;2001; **6**: 840-847

[3] Butler,M.S. The role of natural product chemistry in drug discovery. *J. Nat. Prod.*,2004; **67**, 2141-2153.

[4] Vuorella P, Leinonenb M, Saikkuc P, Tammelaa P, Rauhad JP, Wennberge T, Vuorela H; 2004; Natural products in the process of finding new drug candidates. *Curr Med Chem*; **11**:1375-1389

[5] Koehn FE, Carter GT, 2005; The evolving role of Natural products in drug discovery. *Nat Rev Drug Discov,* **4**:206-220

[6] Osbourn AE, Lanzotti V; Plant derived Natural Products, Synthesis Function and application; *Springer Science;* ; 2009

[7] Yamunadevi Mariswamy, Wesely Edward Gnaraj and Johnson M. Chromatographic finger print analysis of steroids in Areva lanata L by HPTLC technique. *Asian Pacific Journal of Tropical Biomedicine* 2011; 428-433

[8] Kirtikar KR and Basu BD. Indian Medicinal Plant text; 1993;Vol.1

[9] Abraham GJ and Agshikar NV. Anti inflammatory activity of an essential oil from Zanthoxylum budrunga; *Pharmacology PubMed* 1972; **7**(2);109-114.

[10]Agshikar NV and Vaz AX. Local anesthetic action of essential oil extracted from Zanthoxylum budrunga. Ind.3. *Pharmac* 1969; **1**;22-26

[11]Pai V, Savadi RV, Bhandarkar A. Pharmacognostic and phytochemical investigation of stem bark of *Zanthoxylum rhetsa*. *Pharmacog J*, 2009; **1**:33-36

[12]Rahman MT, Alimuzzaman M, Ahmad S, Chowdhury AA. Antinociceptive and antidiarrhoeal activity of *Zanthoxylum rhetsa*. *Fitoterapia*,2002; **73**:340-342

[13]Mathur RK, Ramaswamy SK, Rao AS, Bhattarcharya SC. Isolation of an oxidodiol from *Zanthoxylum rhetsa*. *Tetradron* 1967; **23**:2495-2498

[14]Ahsan M, Zaman TA, Hasan CM, Ito C, Aslam SKN . Constituents and cytotoxicity of Zanthoxylum stem bark. *Fitoterapia* 2000; **71**:697-700

[15]Joy MT, Verghese J, Retamar JA, Talenti ECJ, Catalan CAN, Gros EG . Essential oil of *Zanthoxylum rhetsa*. *Flav Fragran J* 2006; **1**:165-168

[16]Brain K.R and Turner T.D; *The practical evaluation of phytopharmaceuticals* 1975; **98**, 101.

[17]Gyanchandani N.D, Yamamato M, and Nigam I.C: Anthraquinone drugs. I. Thin-layer chromatographic identification of aloes, cascara, senna, and certain synthetic laxatives in pharmaceutical dosage forms.*J. pharm. Sci.* 1969; **58**(2), 197.

[18]Harborne J.B. Phytochemical methods. 3rd Edition London: Chapman and Hall, 1998.

[19]Eike Reich, Anne Schibili . High Performance Thin Layer Chromatography for the Analysis of Medicinal Plants Thieme-New York. Stuttgart; 2007; p. 224-40

[20]Wagner R and Bladt S, Plant Drug Analysis, A Thin Layer Chromatography Atlas, 2nd Ed., Springer, Berlin; 1996.

[21]Wink M; Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective; *Phytochemistry;* 2003; **64**: 3-19

[22]Konig, G.M, Kehraus S, Seibert SF, Abdel-Lateff A and Muller D; Natural products from marine organisms and their associated microbes; *Chembiochem;* 2006; **7**:229-238

[23]Newman DJ, Cragg GM; Natural products as sources of new drugs over the last 25 years. *J Natprod,* 2007; **70**:461-77

[24]Nicolaou KC, Yang Z, Liu JJ, Ueno H, Nantermet PG, Guy RK, Claiborne CF, Renaud J, Couladouros EA, Paulvannan K et al; Total synthesis of Taxol. *Nature,* 1994: **367**:630-4

[25]Kuehne Martin E, Matson Patricia A, Bornann William G. Anantio selective synthesis of Vinblastine, Leurosidine, Vincovaline and 20′-Epivincovaline; *J Org Chem;* 1991; **56**:513-28

[26]Graening T, Schmalz HG;Total synthesis of colchicines in comparison: A Journey through 50 years of synthetic organic chemistry. *Int. Ed*. 2004; **43**;3230

[27]Cook NC and Sammans S; Flavanoids-Chemistry, metabolism, cardioprotective effects and dietary sources. Nutr. *Biochem*.; 1996; **7**;66

[28]Harborne JB, and Williams CA, Advances in Flavanoids research since 1992; *Phytochemistry;* 2000; **55**: 481

[29]Rice-Evans, CA, Miller, NJ and PagangaG. Anti oxidant properties of Phenolic compounds. *Trends Plant Sci.*1997; **2**:152.

[30]Yao, L.H.,Jian Y.M. Shi J, TomasBarberan, FA DattaN, Singanusong R and Chen SS;Flavanoids in food and their health benefits. *Plant Foods Hum. Nutr.;* 2004;**59**;113

[31]R.H. Thomson, Naturally Occurring Quinones, Academic Press, New York, 1971.

[32]E.D. Zanbower, C.M. Kam, J.C. Powers, L.H. Zalkow, *J. Med. Chem.;* 1992; **35**; 1597

[33]Cowan MM. Plant Products as antimicrobial agents. Clin. Microbiol. *Rev.* 1999; **12**(4):564-582.

[34]Wijnsma R, Verpoorte R. Anthraquinones in the Rubiaceae. In: Hill RA, Krebs HCh, Verpoorte R, Wijnsma R, Herz W, Grisebach

H, Kirby GW, Tamm Ch, eds. Progress in the Chemistry of Organic Natural Products. *Wien: Springer-Verlag*;1986, **49**:79-149.

[35]Rath G, Ndonzao M, Hostettmann K. Antifungal anthraquinones from Morinda lucida. Int. *J. Pharmacognosy*. 1995; **33**(2):107-114.

[36]Ali AM, Ismail NH, Mackeen MM, Yazan LS, Mohamed SM, Ho ASH, Lajis NH. Antiviral, cytotoxic and antimicrobial activities of anthraquinones isolated from the roots of Morinda elliptica. Pharm. *Biol*. 2000; **38**(4): 298-301.

[37]Agarwal SK, Singh SS, Verma S, Kumar S. Antifungal activity of anthraquinone derivatives from Rheum emodi. *J. Ethnophamacol.* 2000; **72**:43-46.

[38]Stevic, T.; Savikin, K.; Ristic, M.; Zdunic, G.; Jankovic, T.; Krivokuca, D. and Vulic, T.: Composition and antimicrobial activity of the essential oil of the leaves of black currant (Ribes nigrum L.) cultivar Cacanska crna. *J. Serb. Chem. Soc* 2010; **75** (1): 35- 43.

[39]Meng, K., W.; Liang, Yu.; Sheng, L. and Cheng, E.: Effects of emodin and double blood supplies on liver regeneration of reduced size graft liver in rat model. *World Journal of Gastroenterology* 2005; **11** (19): 2941- 2944.

[40]Cushnie, T.P.T. and Lamb, A.J.: Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 2005; **26**: 343- 356.

[41]Park, B.S.; Lee, H.K.; Lee, S.E.; Piao, X.L. ; Takeoka, G.R.; Wong , R.Y.; Ahn, Y.J. and Kim, J.H.: Antibacterial activity of Tebebuia impetiginosa Martius ex Dc (Taheebo) against Helicobacter pylori. *Journal of Ethnopharmacology* 2006; **105**: 255- 262.

[42]Rios J.L, Recio M.C ; Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*.;2005;**100**:80-84

[43]Cetin H, Erler F, Yanikoglu A. 2004.Larvicidal activity of a botanical natural product, AkseBio2, against Culex pipiens. *Fitoterapia* **75**: 724-728.

[44]Isman MB. 2000. Plant essential oils for pest and disease management. *Crop Protection*;**19**: 603-608.

[45]Amvam Zollo, P.H., L. Biyiti, F. Tchoumbougnang, C. Menut, G. Lamaty and P.H. Bouchet, Chemical composition and antifungal activity of International Rules for Seed Testing. International thirteen essential oils from aromatic plants of Cameroon. *Flavour and Fragrance J*.,;1998; **13**: 107-114.

[46]Isman, M., Pesticides based on plant essential oils. Pestic. *Outlook*;1999;**106**: 68-72.

[47]Nguefack, J., I. Somda, C.N. Mortensen P.H. Amvam Zollo. Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (Oryza sativa L.). Seed Sci. *Technol.;*2005; **33**: 397-407.

[48]Nguefack, J., S.K. Nguikwie, D. Fotio, B. Dongmo, V. Leth, A.E. Nkengfack and P.H. Amvam Zollo,;Fungicidal potential of essential oils and fractions from Cymbopogon citratus, Ocimum gratissimum infection of rice by Helminthosporium oryzae and Thymus vulgaris to control Alternaria padwickii -and Bipolaris oryzae, two seed-borne fungi of rice (Oryza sativa L.). *J. Essential Oil Res*.;2007; **19**: 581-587

[49]Oka, Y., S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv and Y. Spiegel. Nematicidal activity of essential oils and their components against the root-knot and its role in paddy seed spoilage. *Phytopathology;*2000; **90**: 710-715.

[50]Tagne, A., J. Nguefack, C. The and Amvam P.H. Zollo;La lutte naturelle contre les champignons des semences du maïs à l'aide des huiles essentielles et des extraits de plantes. In: Biosciences and Biodiversity. Proceeding of the Annual Conference of the Cameroon Biosciences Society, December 1998, Buea, *Cameroon*, **5**: 230-234.

[51]Wilson, C.L., J.M. Solar, A. El Ghaouth and M.E. Wisniewski. Rapid evaluation of plant extract and essential oils for antifungal activity against Botrytis cinerea. *Plant Disease*, 1997;**81**: 204-210.

[52]Aoife Lacy and Richard O'Kennedy Studies on Coumarins and Coumarin-Related Compounds Current Pharmaceutical Design, 2004, Vol. 10, No. 30 3809

[53]Weber US, Steffen B, Siegers CP. Antitumour-Activities of Coumarin, 7-Hydroxycoumarin and its Glucuronide in Several Human Tumour Cell Lines. *Res Commun Mol Pathol Pharmacol* 1998; **99**: 193-206.

[54]Cooke D. Studies on the Mode of Action of Coumarins (Coumarin, 6-hydroxycoumarin, 7-hydroxycoumarin and Esculetin) at a Cellular Level. PhD Thesis, Dublin City University, Dublin, Ireland, 1999

[55]Egan D, James P, Cooke D, O'Kennedy R. Studies on the Cytostatic and Cytotoxic Effects and Mode of Action of 8-Nitro-7-Hydroxycoumarin. *Cancer Letts* 1997; **118**: 201-211.

[56]Cooke D, O'Kennedy R. Comparison of the Tetrazolium Salt Assay for Succinate Dehydrogenase with the Cytosensor Microphysiometer in the Assessment of Compound Toxicities.*Anal Biochem* 1999; **274**, 188-194

[57]Sam Zwenger and Chhandak Basu* Biotechnology and Molecular Biology Reviews; 2008; Vol. **3** (1), pp. 001-007

[58]Islam AK, Ali MA, Sayeed A, Salam SM, Islam A, Rahman M, Khan GR, Khatun S;An antimircrobial terpenoid from Caesalpinia pulcherrima Swartz.: Its characterization, antimicrobial and cytotoxic activities. *Asian J. Plant Sci.;* 2003; **2**: 17-24.

[59]John AJ, Karunakran VP, George V. Chemical composition an antibacterial activity of Neolitsea foliosa (Nees) Gamble var. caesia (Meisner) Gamble. J. *Essent. Oil Res*.; 2007; **19**: 498-500.

[60]Murata T, Miyase T, Muregi FW, Naoshima-Ishibashi Y,| Umehara K, Warashina T, Kanou S, Mkoji GM, Terada M, Ishih A ;Antiplasmodial triterpenoid from Ekebergia capensis. *J. Plant Nat. Prod.* 2008; In press

[61]Newman DJ, Cragg GM, Holbeck S, Sansville EA. Natural products and derivatives as leads to cell cycle pathway targets in cancer chemotherapy; *Current cancer drug targets;* 2002; **2**; 279-308

[62]Koehn FE. Thrapeutic potential pf Natural product signal transduction agent; *Current Opin Biotechnol;* 2006; **17**; 631-637