IJBPAS, September, 2022, 11(9): 4383-4397

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A REVIEW: PHARMACOGNOSTIC AND BIO-CHEMICAL CHARACTERIZATION OF *JUSTICIA ADHATODA* L.IN LOWER SHIVALIK HILLS OF HIMACHAL PRADESH (INDIA)

KUMAR G[1*], RAWAT DS[2], KHARWAL AD[3], KUMAR N[4] AND RAWAT S[5]

1: Division Botany, Department of Bio-Sciences, Career Point University Hamirpur, Himachal Pradesh- 176041, India

2, 4, 5: Department of Bio-Sciences, Himachal Pradesh University, Shimla, India (171005)

3: Department of Botany, Post Graduate College, Dharamshala (Himachal Pradesh), India

***Corresponding Author: Dr. Gulshan Kumar: E Mail: sharmagulshan1980@gmail.com**

Received 16th Oct. 2021; Revised 20th Nov. 2021; Accepted 20th Jan. 2022; Available online 1st Sept. 2022

https://doi.org/10.31032/IJBPAS/2022/11.9.6429

ABSTRACT

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human diseases. A plant derived drug serves as a prototype to develop more affective and less toxic medicine. The sources of indigenous medicines have not been studied extensively. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products. The evaluation of plant products on the basis of medicinal and therapeutic properties forms a platform for the discovery of newer drug molecules from different plant sources. *Justicia adhatoda* L. *is* well known in the indigenous system of medicine for its beneficial health effects, particularly in treating bronchitis. The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol and water-soluble extractives were determined as per standard methods. Air-dried powdered material has been subjected to qualitative and quantitative physicochemical estimations as per standard methods.The findings of extractive values revealed that it contains higher amount of highly water soluble bio-constituents **[51]**. The study revealed that the leaves of *Justicia adhatoda* L. contain

higher amount of semi polar and polar secondary metabolites as well as the pharmacological activity of plant material varies according to its polarity or nature of bio-chemical constituents. The present study aims to compile medicinal values of *Justicia adhatoda* L. generated through the research activity using modern scientific approaches and innovative scientific tools.

Keywords: Pharmacological, Pharmacognostic, Therapeutic, Indigenous medicines, Shivalik hills

INTRODUCTION

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human diseases. These plants are the backbone of traditional medicines in the last few decades and is a subject of very intense pharmacological studies **[1]**. Medicinal plants are the as potential source of new compounds of therapeutic value in drug development **[2]**. In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral activities. A plant derived drug serves as a prototype to develop more affective and less toxic medicine. The sources of indigenous medicineshave not been studied extensively **[3]**. The Shivalik zone that lie in the districts Hamirpur, Bilaspur, Una, Mandi, Sirmour and Solan regions,is an extensive abode of the tribal communities such as *Kohlies* and *Gujjars* **[4]**. They are semi-nomadic, semi-pastoral and semi-agriculturalist type **[5]**. This range is considered as the richest source of traditional and potential medicinal wealth. In ancient times, it had been the abode of *rishies* and *munies* (prophet, sages or saints), who pursued their meditational and scholarly endeavors here **[6]**. The knowledge acquired by few local inhabitants, descended down from generation to generation still forms the traditional practice of herbal cure **[7]**. Several screening studies have been carried out in different parts ofthe world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world **[8]**. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products **[9]**. Now-a-days, there is manifold increase in medicinal plant based industries **[10]**. Despite the major advances in the modern medicine, the development of new drugs from natural products is still considered important. Traditional therapeutics based on herbal medicinal principles is time tested and widely accepted across various cultural and socioeconomic strata **[11]**. However, there is lack of precise guidelines to study the herbal compounds and till date a very meagre portion of this tremendous knowledge has

been scientifically screened. Hence, there is a real need for scientific evidence based validation of these agents **[12]**. The evaluation of plant products on the basis of medicinal and therapeutic properties forms a platform for the discovery of newer drug molecules from different plant sources. *Justicia adhatoda* L.

Justicia adhatoda L. is an aromatic large shrub of about 1.5 meter in height and itis a perennial, evergreen and highly branched with unpleasant smell and bitter taste. It has opposite ascending branches with white, pink or purpleflowers. It is found in moist area, often on banks of riversup to an altitude of 1,500 meter. Various medicinal properties are attributed to it *e.g.*anti-inflammatory, against fungal diseases, antioxidant and hepatoprotective disorders. *Justicia adhatoda* L., commonly known as the 'Basuti' in lower Shivalik hills of Himachal Pradesh **[13]**. Herbal remedies are a type of alternative medicine that originates from plants and plant extracts, used to heal illnesses and diseases and were the precursor to modern medicine. Herbal remedies are obtained from a wide variety of natural resources including plant leaves, flowers and roots. "Vasaka" has been used traditionally by herbalists and indigenous healers worldwide for the prevention and treatment of liver disease and has potent anti-periodic, astringent, diuretic and purgative action also used against asthma, cough, bronchitis and tuberculosis **[14]**. The whole plant has antispasmodic property. The activities against tuberculosis were reported by many researchers quite early. It has been used extensively as an important herbal drug in treating a wide variety ofdiseases. 'Vasaka' is well known in the indigenous system of medicine for its beneficial health effects, particularly in treatingbronchitis.In lower Shivalik, *Justicia adhatoda* L.has been used in the treatment of cold, cough,pneumonia, fever, jaundice, catarrh, whooping cough and asthma **[15]**. Basic scientific research has uncovered the mechanisms by which some plants afford their therapeutic effects. In recent years many researchers have examined the effects of the plants used traditionally by indigenous healers and herbalists to support lung function and treat diseases of the respiration. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and mode of action of these plants, as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies **[16]**.

The plant has a pungent, bitter and acrid taste; good as an astringent, stomachic, anthelmintic; promotes the growth of hair;

useful in inflammation, leucoderma, bronchitis, asthma and even chronic tuberclosis. The root is anantidote to scorpion bite. The plant is reported to haveanodyne, antiseptic, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic and vulnerary **[17]**. A decoction of 'Basuti' leaves is given with the addition of long pepper (*Piper longum*) in catarrhal fever. A pillow stuffed with the leaves of 'Basuti' is placed under the head for relief of headache. The juice of the leaves is said to have the property of removing foetid discharges and worms from ulcers **[18]**. The flowers are useful in diarrhoea, cholera, fever, haemorrhages, and respiratory disorders. Tincture of root bark (two drops) is recommended in cases of irritable bladder and rheumatism. Powdered root is prescribed for piles as a demulcent for dysentery. Root is used in dyspepsia, colic, rheumatism, worms, boils and leprosy **[19]**.

METHODOLOGY

Authentication:

The fresh leaves of *Justicia Adhatoda* L. of acanthaceae family was collected from Hamirpur district of Himachal Pradesh, India and authenticated by professor Dr. P.K. Sharma, Scientist, KVK-Kangra, CSKHPKV Palampur, Himachal Pradesh (India) May, 2018 and the registered Number of the Specimen is CPU/2018/2002.

Justicia Adhatoda **L. (Basuti); Location-Bhota chowk, Hamirpur**

Assertion Number of the Specimen is CPU/2018/2002

Preparation of Extracts:

Physical and Pharmacognostic evaluation: The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol and water-soluble extractives were determined. Air-dried powdered material has been subjected to qualitative and quantitative physicochemical estimations.The obtained extracts were stored in desiccators for further phytochemical and antimicrobialinvestigations. The dried material was tested for its constituents by standard methods **[20-22]**.

Ash values: Three gms of air dried powdered drug has been taken in a silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon and then weighed, repeated for constant value. Then the percentage of total ash has been calculated with reference to the air dried drug.

a) Analysis of acid insoluble ash value: The total ash has been boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter has been composed on a filter paper, washed with hot water, ignited and weighed to calculated the percentage of acid insoluble ash with reference to the air dried drug **[23]**.

b) Analysis of water soluble ash value: The total ash obtained has been boiled with 25 ml. of water for 5 minutes. The insoluble matter has been collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at 450°C. The percentage of water soluble ash has determined with the air dried drug. Cooling is done in desiccators and the percentage of total ash is calculated from the weight of the drug taken **[24]**.

Physical and Pharmacognostic evaluation: The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and water-soluble extractives were determined. Air-dried powdered material has been subjected to qualitative and quantitative physicochemical estimations **[25-26]**.

Weight loss on drying: Loss on drying is the loss of weight in % w/w by weighing 1.5 gm. of powdered drug in a tared porcelain dish, which has previously dried at 105°C in hot air oven. The percentage loss of drying to the air dried substance has been calculated **[27]**.

Extractive values of crude drug: Extractive values of crude drugs are functional for their appraisal and these values identify the nature of the constituents present in a crude drug. Five gms of the air-dried coarse powder of the plant material was macerated with 100 ml of 90% ethanol in a closed flask for twenty four hours with gentle shakingfor the first six hours and then allowing to stand for 16 hours **[28]**. Thereafter, it was filtered and dried at

105 °C and weighed. The percentage of ethanol and water soluble extractive was calculated with the air-dried drug **[29]**.

Fluorescence analysis: Fluorescence characteristics of the powdered plant material have been analyzed in daylight and UV light **[30]**.

Preliminary biochemical studies:

Extract prepration: The powdered leaves are keeping separately in soxhlet apparatus and again treated with petroleum ether, hydroalcohol (mixture of 70% ethanol and 30% distilled water) and distilled water. The resultant was distilled in vacuum under low pressure and dried in a desiccator **[31]**.

Qualitative chemical analysis: Qualitative chemical tests have been performed to determine the presence of alkaloids, carbohydrates, protein, cardiac glycosides, polyphenols, saponins and terpenoids **[32]**.

Alkaloids analysis:

(i) Dragendorff's test: To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicated the presence of alkaloids **[33]**.

(ii) Mayer's test: To 1 ml of the extract, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish green or cream colored precipitate indicated the presence of alkaloids **[34]**.

Proteins analysis:

Millon's test: 1ml of test solution was acidified with sulphuric acid and mixed with Millon's reagent and boiled to get yellow precipitate which indicate the presence of protein **[35]**.

Glycosides analysis:

Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered. To the filtrate, 10ml of water with 0.5ml of strong solution of lead acetate was added and the filtrate was gently shaken with 5ml of chloroform. The chloroform layer is alienated in a porcelain dish and solvent was separated by gentle evaporation. The cooled residue was dissolved in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution and shifted to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer formed at the junction of the two liquids withbluish green upper layer which slowly turned darker.

Carbohydrates analysis:

Molisch's test: To 2ml of the extract, 1ml of α-napthol solution with concentrated was added and the purple or red colour at the junction of the two liquids revealed the presence of Carbohydrates **[36]**.

Fehling's test: To 1ml of the extract, equal quantities of Fehling solution A and B was

added which upon heating led to the formation of a brick red precipitate indicating the presence of sugars **[37]**.

Phenolic compounds analysis: The extract is treated with Potassium ferric cyanide and NH_4 solution and a deep red colour indicated the presence of tannins **[38]**.

Flavonoids Analysis:

(i) **Shinoda's test:** The alcoholic extract tested with magnesium (Mg) foil and concentrated HCl furnished passionate cherry red colour which showed the presence of flavonones and orange red colour indicated the presence of flavonols **[39]**.

(ii) The extract is treated with NaOH and the formation of yellow colour indicated the presence of flavones **[40]**.

(iii) The extract is treated with concentrated H_2SO_4 resultes in yellow or orange colour for flavones **[41]**.

Steroids analysis:

Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4 make of bluish red to cherry colour in chloroform layer as well as green fluorescence in the acid layer indicates the steroidal components **[42]**.

Saponin analysis:

(i) **Spot Test:** Press a small quantity of extracts between the filter paper and oil stains on paper represents the presence of fixed oils **[43]**.

(ii) **Saponification test:** To 1ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein as well as water bath it for 1-2 hours. The soap formation of 1 cm layer shows the presence of fixed saponins **[44]**.

RESULTS AND DISCUSSION

Propagation and Planting: For commercial production, seeds are sown in a seed tray in mid spring and then planted. *Justicia adhatoda* L. is used as a ethno- medicinal plant and is not a part of trade chain in the international market **[45]**. It is cosmopolitan and globally inhabited in Temperate-Asia (China, Guangdong, Hunan, Sichuan, Yunnan, Zhejiang, Eastern Asia, Japan) Tropical-Asia (Nepal, Indo-China, Burma, Thailand, Vietnam, Malesia, Jawa, Papua New Guinea, Philippines), Australia and Sri Lanka Pacific **[46]**. It is found in Indian subcontinent (Assam, Bangladesh, Bihar, Maharashtra, Orissa, Punjab, Tamil Nadu, Uttar Pradesh) **[47]**. In Himachal Pradesh, It is frequently found in Una, Hamirpur, Bilaspur, Mandi, Kangra, Solan and Sirmour **[48]**.

Ethno-medicinal Remediation: Itis used mostly against bronchitis and *influenza* infections. It is also generally used in cough,

common cold and bronchitis as well as in respiratory disorders. Its decoction is mostly used in Leucorrhoea, Cough, Tuberclosis, Jaundice, Vomting, Diuretic, and Stomach disorders, pain in the joints, insect or snake bites, and *Cannabis sativa* poisoning in lower shivallik hills of Himachal Pradesh **[49]**. In India, the herb is boiled to a paste and applied to the cheeks for toothache, and also used for other swellings and inflammations.

Pharmacognostical studies: The efficiency and accuracy of pharma products are depended on its extraction by applying standard operating procedure. The physicochemical and bio-chemical characters of *Justicia adhatoda* L. were studied and the results are presented in **Tables 1 [50]**. Inorganic materials, such as carbonate, silicates, oxalates, and phosphates were analysed through its total ash-value (5.2%). The inorganic components were extracted by heating organic components with the release of CO_2. The findings of extractive values revealed that it contains higher amount of highly water soluble bio-constituents **[51]**. The study revealed that the leaves of *Justicia adhatoda* L. contains higher amount of semi polar and polar secondary metabolites as well as the pharmacological activity of plant material varies according to its polarity or nature of bio-chemical constituents **[52]** . Two and half hundred grams of crude dried powder of leaves of *Justicia adhatoda*L.was extracted successively with petroleum ether (70-75°C), chloroform, ethyl acetate and methanol in soxhlet extractor for thirty six hours **[53]**. Greenish residues were obtained after concentrating the extract under low pressure (Yield 7.90%, 3.50%, 1.85% and 14.00% respectively). Unless specified the dried plant material is grounded in a Waring blender and then extracted in the specified solvents by steeping overnight at room temperature. The resulting extracts are then filtered through a glass frit and evaporated to dryness on a rotary evaporator. The preliminary screening of each plant is performed by LC-MS. MilliQ (MQ) water, Liquid Chromatography-Mass Spectrometry (LC-MS) **[54]**. Phytochemical components in petroleum ether extract of *Justicia adhatoda* L. by LC-MS report. The LC-MS analysis revealed the presence of nine compounds from the petroleum ether leaf extract. The major constituents were *9-octadecenoic acid ethyl ester, Heptadecanoic acid, 15-methylethylester, 16-octadecenoic acid, methyl ester* along with other minor constituents were also present. The compounds that are responsible for diagnostic effect are usually the secondary metabolites **[55-59]**. The bio-chemical study

of leaves extract of *Justicia adhatoda* L.exposed the presence of triterpenoids, alkaloids, anthraquinones, flavonoids, saponins, biosterols, triterpenoids and poly-phenols **(Table 1.0)**. Carbohydrates were absent in all the extracts. Physicochemical parameters and extractive value of leaves revealed the results as moisture 15.20% (leaves) and the total ash content, acid insoluble ash, Water soluble ash and foreign matter values which were determined to be not more than 11.40%, 1.50%, 3.85%, 1.68% and compared with present proximate analysis value with menthol solvent (14.00%) respectively **[60-64]**.

Table 1: Bio-chemical screening analysis of various extracts of *Justicia adhatoda* L. leaf

Analytical Test	Analytical test	Petroleum Ether	Ethyl Acetate	Chloroform	Methanol
Alkaloids	Dragendorff's test	-	+	+	+
	Mayer's test	-	+	+	+
Anthraquinones		-	+	-	+
Carbohydrates	Molisch's test	-	-	-	-
	Fehling's test	-	-	-	-
Flavonoids	Shinoda's test	-	+	+	+
Saponins		-	+	-	+
Biosterols		+	+	-	+
Triterpenoids		+	+	+	+
Proteins	Millon's test	-	-	-	+
Glycosides analysis	Keller-Killiani test	-	-	-	+
Phenolic compounds	Potassium ferric cyanide test	-	+	-	+
Steroids	Salkowski test	-	+	-	+

CONCLUSION

The systematic study on *Justicia Adhatoda* L. showed its ethnomedicinal and pharmacognostic importance as well as an alternative for primary health care system in lower Shivallik hill regions of Himachal Pradesh. The natural stock of medicinal plants is under tremendous pressure, since, must be conserved and promoted its commercial cultivation. It possesses numerous biological activities proved by many experimental studies. It represents a class of herbal drug with very strong conceptual base for its use. Thus, this plant has great potential to be developed as a drug by pharmaceutical industries, but before its recommendation for clinical use in these conditions, there is a need to conduct clinical trials and prove its clinical utility. Medicinal plants, which are the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies. There arises a need therefore to screen medicinal plants for bioactive

compounds as a basis for further pharmacological studies.Pharmacognostic and bio-chemical investigation also provides useful information in this regard to its correct identity and help to differentiate it from its other closely related species. The present study aims to compile medicinal values of *Justicia adhatoda* L. generated through the research activity using modern scientific approaches and innovative scientific tools.

REFERENCES

[1] Paarakh PM. *Nigella sativa* Linn.-A comprehensive review.Indian Journal of Natural Products and Resources, 1(4), 2010; 409-429.

[2] Sahare KN, Anandhraman V, Meshram VG, Meshram SU,Reddy MVR, Tumane PM and Soswami K. Anti-microfilarial activity of methanolic extract of *Vitex negundo* and *Aegle marmelos* and their phytochemical analysis. Indian Journal of Experimental Biology, 46, 2008; 128-131.

[3] Pandey MM, Rastogi S and Rawat, A.K.S. Indian herbal drug for general healthcare: an overview. The Internet Journal of Alternative Medicine, 6(1), 2008; 1-3.

[4] Reddy YSR, Venkatesh S, Ravichandran T, Subbaraju T and Suresh B. Pharmacognostical studies of *Wrightia tinctoria* bark. Pharma Biol., 37, 1999; 291- 295.

[5] Rastogi RP and Mehrotra BN, Compendium of Indian Medicinal Plants, Vol I–V, Central Drug Research Institute, Lucknow and National Institute of Science Communication, New Delhi, India, 1994.

[6] Asolkar LV, Kakkar KK and Chakra OJ, Second Supplement to Glossary of Indian Medicinal Plants with active principles, Part I Publication and information Directorate (CSIR), New Delhi, India, 1992.

[7] Harborne JB, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, New York: 3rd Edition, 1973.

[8] Anonymous. The Wealth of India, Raw Materials. Vol.10.New Delhi: CSIR (Council of Scientific & Industrial Research) 2003; 158-160.

[9] Ayurvedic Pharmacopoeia of India. Part 1, Vol.3, NewDelhi, Government of India Publication 2001; 142-144.

[10] Kirtikar KR and Basu BD. Indian medicinal plants. Text Vol.3, International Book Distributors, Dehradun 2008 (latest edition); 1937-1940.

[11] Kumar G. and Chander H. Study on the Potential of *Azolla pinnata* as Livestock Feed Supplement for Climate Change Adaptation and Mitigation.Asian J. Adv. Basic Sci., 5(2), 2017; 65-68.

[12] Kumar G. and Chander H. Polylined water harvesting tank technique to mitigate the impact of climate change on agro-economy in rain fed conditions: A case study, J. Biol. Chem. Chron., 4(1), 2018; 01-07.

[13] Kumar G. and Chander H. Documentation of indigenous agricultural implements, practicesand other conservation techniques in subtropical climatic zone of Shivalik hills, North Western Himalaya, J. Biol. Chem. Chron., 3(2), 2017; 15-23.

[14] Kumar G. and Chander H. Ethno-Veterinary and fodder plants of Awah-Devi region of Hamirpur district, Himachal Pradesh, J. Biol.Chem. Chron., 4(1), 2018; 08-15.

[15] Kumar G. and Chander H. (2018). Polylined Water Harvesting Tank Technique to Mitigate the Impact of Climate Change on Agro-economy in Rain Fed Conditions: A Case Study, J. Biol.Chem. Chron., 4(1), 2018; 01-07.

[16] Nadkarni KM. Indian Materia Medica. Vol. 1: Bombay Popular Prakashan 2002; 1278-1280.

[17] Ayres DC and Loike J D. Lignans: Chemical, Biological and Clinical Properties; Cambridge University Press: Cambridge, 1990.

[18] Barr A. Traditional Bush Medicines: An Aboriginal Pharmacopoeia. Aboriginal Communities of Northern Territory Australia. Greenhouse Publications. Richmond, Victoria, 1988.

[19] Barrero AF, del Moral QJ and LaraA. Sesquiterpenes from *Juniperus thurifera* L. Stereochemistry in Undual Cedrane and Duprezianane series. Tetrahedron, 56, 2000; 3717-3723.

[20] Liang H, Bao F, Dong X, Tan R, Zhang C, Lu Q and Cheng Y. Antibacterial Thymol Derivatives Isolated from *Centipeda minima*. Molecules, 12, 1974; 1606-1613.

[21] Liu ZG, Yu HM, Wen SL and Liu YL. Histopathological study on allergic rhinitis treated with *Centipeda minima*. Zhongguo Zhong Yao Za Zhi, 30(4); 2005; 292-294.

[22] Kumar T and Chandrashekar K.S. *Bauhinia purpurea* Linn.: A Review of its Ethnobotany, Phytochemical and Pharmacological Profile. Research

Journal of Medicinal Plants, 5, 1974; 420-431.

[23] Sharma PC, Yelne MB and Dennis T.J. Database on MedicinalPlants Used in Ayurveda. CCRAS Publication, Vol. 3, New Delhi: Reprint Edition 2005; 450-471.

[24] Gupta AK, Tandon N and Madhu S. Quality Standards of Indian Medicinal Plants. Indian Council of Medical Research, Vol. 3, New Delhi 2008; 130-135.

[25] Basu NK and Singh GB. A Note on the Chemical Investigation of *Vitex negundo* L. The Indian Journal of Pharmacy, 6, 1974; 71-74.

[26] Joshi V. Namboori K and Vaghani DD. Chemical components of some Indian medicinal plants. IndianJournal of Chemistry, 12, 1974; 226.

[27] Misra GS and Subramanian PM, Three New Flavones Glycosides from *Vitex negundo*. Journal of Medicinal Plant Research, 38, 1980; 155-160

[28] Gu G. Liu Y and Sun Y. Lipid constituents of *Mujmingzi. Zohong caoyao* 17(10), 1986; 473.

[29] Li S. and Guan S. Research of the inclusion compounds of Chinese medicinal volatile oils with β-cyclodextrin. Zhongyao Tongbao, 12(12), 1987; 731-736.

[30] Chandra S. and Babber S. Synthesis of 5, 4'-dihydroxy-7, 8, 3',5'-tetramethoxyflavone and two new isomericpentaoxygenated flavanones isolated from *Lepidium sativum* and *Vitex negundo*. Indian Journal of Chemistry. 1987; 26B (1), 82-84.

[31] Banerji J. Das B and Chakrabarty R. Isolation of 4, 4'-Dimethoxy-Trans-stilbene & Flavonoids from Leaves & Twigs of *Vitex negundo* Linn. Indian Journal of Chemistry. 1988; 27B, 597-599.

[32] Muralikrishna KS, Latha KP, Shreedhara CS, Vaidya VP and Krupanidhi AM. Effect of *Bauhinia purpurea* Linn. on alloxan-induced diabetic rats and isolated frogs heart. Int. J. Green Pharm. 2, 2008; 83-86.

[33] Marimuthu K and Dhanalakshmi R. A Study on Phytochemicals in *Bauhinia purpurea* L. Leaf and Flower. Int. J. Pharm. Sci. Rev. Res. 29(2), 2014; 72-76.

[34] Boonphong S, Puangsombat P, Baramee A, Mahidol C, Ruchirawat S and Kittakoop P. Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial,

antifungal, anti-inflammatory and cytotoxic activities. J. Nat. Prod. 70, 2007; 795-801.

[35] Zakaria ZA, Rahman NIA, Loo YW, Ayub AHA and Sulaiman MR. Antinociceptive and anti-inflammatory activities of the chloroform extract of *Bauhinia purpurea* L. (Leguminosae) leaves in animal models. Int. J. Trop. Med. 1, 2009; 140-145.

[36] Mukherjee PK. Quality Control Herbal Drugs–An Approach to Evaluation of Botanicals. Business Horizons Pharmaceutical Publishers. New Delhi. 2005; 131-196.

[37] Harbone JB. Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis. 3rd ed. Springer, New Delhi. 2005, 1-32.

[38] Kandelwal KR, Pawar AP, Kokate CK and Gokhale SB. Practical Pharmacognosy Technique and Experiments, 3rd edition, Nirali Prakashan. 1996: 2-4.

[39] Pratt RT and Chase CR. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of American Pharmaceutical Association, 38, 1949; 324-331.

[40] Kokoshi CJ, Kokoshi RJ and Sharma FT. Fluorescence of powdered vegetable drugs under ultra-violet radiation, Journal of American Pharmaceutical Association. 47, 1958; 715.

[41] Dayal R and Singh V. A comparative study of volatileconstituents of *Vitex negundo* leaves. Journal of Medicinal and Aromatic Plant Sciences, 22(I B), 1989: 639-640.

[42] Bhargava SK. Antiandrogenic effects of a flavonoids-richfraction of *Vitex negundo* seeds: a histological andbiological study in dogs. Journal of Ethnopharmacology. 27(3), 1989; 327-339.

[43] Telang RS and Chatterjee S, Studies on analgesic and anti-inflammatory activities of *Vitex negundo*. Indian Journal of Pharmacology. 31, 1999; 363-366.

[44] Krishna V. Verma S, Pareek RB, *et al*. Terpenoidsconstituents from some indigenous plants. Journal of theIndian Chemical Society 79(6), 2002; 550-552.

[45] Diaz F. Chavez D, Lee D, *et al*. Cytotoxic Flavone Analouges of Vitexicarpin, a constituent of the leaves of *Vitex negundo*. Journal of Natural Product 66(6), 2003: 865-867.

[46] Chandramu C, Manohar RD, David Krupadanam GL and Dashavantha RD. Isolation, Characterization and Biological Activity ofBetulinic acid and Ursolic acid from *Vitex negundo* L. Phytotherapy Research. 17, 2003; 129-134.

[47] Haq A and Khan SB. Flavonoid glycoside and a long chainester from the roots of *Vitex negundo*. Polish Journal of chemistry, 78, 2004; 1851-1856.

[48] R Dayal. Phytochemistry of some useful forest plants. Indian Forester, 130(4), 2004; 456- 460.

[49] Prabhakar A. Gupta BD, Suri KA, Datta B, SattiNK, Malhotra S, Johri, RK and Jaggi BS. Hepatoprotective activity of 2'-p hydroxybenzoyl mussaenosidic acid. Patentno. WO 2003 094946.

[50] Prabhakar A. Gupta BD, Suri KA, Datta B, Satti NK, Malhotra S, Johri, RK and Jaggi BS. Hepatoprotective activity of 10-O-p-hydroxybenzoylaucubin. Patent No. WO2003 094911.

[51] Rastogi RP and Mehrotra BN. Compendium of Indian Medicinal plants. CDRI (Central Drug Research Institute, Lucknow) and NISCAIR (National institute of science communication and information resources, New Delhi), (2), 2006; 704.

[52] Rastogi RP and Mehrotra BN. Compendium of IndianMedicinal plants. CDRI (Central Drug Research Institute, Lucknow) and NISCAIR (National institute of science communication 50) and information resources, New Delhi), (3), 2004; 676-678.

[53] Anonymous. The Wealth of India - A Dictionary of Indian Raw Materials & Industrial Products, First Supplement, CSIR (Council of Scientific & Industrial Research, New Delhi), 2004; 342-344.

[54] Prajapati P and Sharma K. A handbook of medicinal plants -A complete source book. Section-II, 1977.

[55] Sharma SS. Gill K, Malik MS and Malik OP. Insecticidal, Antifeedent and growth inhibitory activities of essential oils of some medicinal plants. Journal of Medicinal & Aromatic Plant Sciences, 22(4A)-23(1A), 1997; 373-377.

[56] Shin KH, Chi HJ, Sung LM, Cho SH, Moon HI, Yu JH, Soon S and Hyung I. Antimicrobial activities of volatile essential oils from Korean aromatic plants. Natural Product Sciences, 3(2), 1997; 141-147.

[57] Lee CK. Kim H, Moon KH and Shin KH. Screening and isolation of antibiotic resistance inhibitors from herb material resistance inhibition of volatile

components of Korean aromatic herbs. Archives of Pharmacal Research, 21(1), 1998; 62-66.

[58] Lee CK. Kim H, Moon KH and Shin KH Screening and isolationof antibiotic resistance inhibitors from herb material resistance inhibition of volatile components of Koreanaromatic herbs. Archives of Pharmacal Research, 21(2), 1998; 223.

[59] Iqbal Z. Shaheen M, Hussain F, Sheraz B, Mohammad I, Sahida Z and Bashir A. Anti fungal properties of some indigenous plants from Peshawar Valley. Asian Journal of Plant Sciences, 1(6), 1987; 708-709.

[60] Ahmad MB. Rauf A and Osman SM, Physico-chemicalanalysis of seven seeds oils. Journal of the oil techn-ologist's Association of India, 21(3), 1987; 46-47.

[61] Samy RP. Ignacimuthu S and Sen A, Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology, 62, 1987; 173-182.

[62] Rimando MA. Ino Shiri OS, Hideaki O, Kohda H, Kazuo Y, William PG, Lelani T, Erneste QG and Magdalena CC. Screening for mast cells histamine release inhibitory activity of Philippine Medicinal plants. Japanese Journal of Pharmacognosy. 41(3), 1987; 242-247.

[63] Chaturvedi GN and Singh RH. Experimental studies on antarthritic effect of certain indigenous drugs, *Indian J medres*, 53 (1), 1965; 71-80.

[64] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants (Publication and Information Directorate, Council of Scientific and Industrial Research, New Delhi), 1956; 256-257.