International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 8, Issue 2, 2016

Review Article

A REVIEW ON PALMYRA PALM *(BORASSUS FLABELLIFER)*

VEDA PRIYA GUMMADI*[1], GANGA RAO BATTU[2], KEERTHANA DIYYA M. S[3], KIRAN MANDA[4]

***A. U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A. P, India 530003, [1,2,3,4]A. U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A. P, India**
Email: gummadi.veda88@gmail.com

Received: 11 Feb 2016, Revised and Accepted: 19 Mar 2016

ABSTRACT

The medicinal plants have very important role in the health of human beings as well as animals. India is the largest producer of medicinal plants. One such plant, *Borassus flabellifer* L, belongs to family Arecaceae, commonly known as Palmyra palm is a native of tropical Africa but cultivated throughout India. Traditionally the different parts of the plant such as root, leaves, fruit, and seeds are used for various human disorders. Leaves are used for thatching, mats, baskets, fans. Flowers of *B. flabellifer* were investigated for analgesic and antipyretic effects, anti-inflammatory activity, haematological, biochemical parameters, and immunosuppressant property. The different parts of the plant are being used for medicinal properties like antihelminthic and diuretic. The fruit pulp of *B. flabellifer* has been used in traditional dishes and the sap, has been used as a sweetener for diabetic patients. Phytochemical studies of the plant revealed the presence of spirostane-type steroid saponins; steroidal glycoside also contains a bitter compound called flabelliferrins. Although investigations have been carried out a lot more can still be explored, exploited and utilized. The present review highlights the phytochemical and pharmacological studies including folklore medicinal uses of this plant.

Keywords: *Borassus flabellifer*, Traditional uses, Phytochemistry, Pharmacology.

INTRODUCTION

Plants have been used for health and medical purposes for several thousands of years. Medicinal plants play a key role in the human health care system. Knowledge on the Medicinal plants provides a new way for modern drugs development [1]. Herbal medicines are in great demand in the developed world of primary health care because of their safety, efficacy and lesser side effects. Out of these, the real medicinal value of over 4,000 plants is either little known or unknown to the mainstream population [2]. Herbal medicines have become more sensitive to unwanted effects over synthetic medicines which led to the increasing demand for herbal resources and awareness for maintaining quality and purity of raw materials [3]. Herbal medicines are popular remedies for diseases used by a vast majority of the world's population [4]. The healing properties of many herbal medicines have been recognized in many ancient cultures [5]. In this scenario, detailed review of this plant *Borassus flabellifer* has been discussed.

Classification [6]

Kingdom: Plantae

Sub-Kingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Arecidae

Order: Arecales

Family: Arecaceae

Genus: *Borassus* L.

Species: *Borassus flabellifer* L.

Synonyms [7]

Wine Palm, longer palm, lontar, palmyra, toddy palm.

Vernacular names [8, 9]

Hindi: Taad

English: Toddy palm, Palmyra palm

Tamil: Talam

Telugu: Tatichettu

Malayalam: Karimpana

Bengali: Taala

Kannada: Olegari

Sanskrit: Taalah

Botanical description

Borassus flabellifer L, belongs to family Arecaceae, commonly known as Palmyra palm is a native of tropical Africa but cultivated and naturalized throughout India [10]. It is a robust tree and can live more than 100 y and reach a height of 30 metres (98 ft), with a canopy of green-bluish leaves with several dozen fronds spreading 3 m (9.8 ft) across. Leaves are used for thatching, mats, baskets, fans, hats, umbrellas, and base of young leaf stalks is used for straining the Toddy and for making torches [11]. Flowers of *B. flabellifer* were investigated for analgesic and antipyretic effects [12] anti-inflammatory activity, haematological and biochemical parameters [13, 14] immunosuppressant property [15]. Pellets of *B. flabellifer* Linn. showed a significantly reduced capacity to mount a delayed-type hypersensitivity (DTH) [16] and flour from the young shoots of the *B. flabellifer* tested for mutagenicity [17], mitogenic activity [18], neurotoxic effect [19]. The fruit measures 4 to 7 inches in diameter; The fruits are large and fibrous, containing usually three nuts like portions each of which encloses a seed [20].

The stem of the leaves has thorny edges. Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin. It also contains 20 known steroidal glycosides [21] and carbohydrates like sucrose [22]. It also contains a bitter compound called flabelliferrins; these are steroidal saponins. *B. flabellifer* contains gums, albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C [23]. The fresh sap is a good source of vitamin B-complex [24].

The different parts of the plant are being used for medicinal properties like antihelminthic and diuretic [25, 26]. The fruit pulp of *B. flabellifer* has been used in traditional dishes and the sap, has

been used as a sweetener for diabetic patients [27]. *Borassus* is a genus of six species of fan palms, fruits are eaten either roasted or raw, and the young, jellylike seeds are also edible [28].

Phytochemistry

Phytochemical screening was performed in all the extracts [29]. Alkaloids test was performed by Mayer's tests, amino acids by ninhydrin, carbohydrates by barfoed's and fehling tests, flavonoids by $FeCl_3$, glycosides by Legal test, saponin by alcoholic vanillin test, tannins by $FeCl_3$ and lead acetate & triterpenoids by Libermanan-Burchard's test [30, 31]. The tests for tannins, phenols, were also carried out as per standard protocols.

Borassus flabellifer contains albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C [32]. The fresh sap is reportedly a good source of vitamin B-complex [33]. Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin [34]. It also contains 20 known steroidal glycosides and carbohydrates like sucrose. It also contains a bitter compound called flabelliferrins; these are steroidal saponins [35]. 28 chemical constituents have been identified from ethanol root extract of *Borassus flabellifer* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis namely 2-Furanmethanol, Propane, 1-(1-methylethoxy), 2-Cyclopenten-1-one, 2-hydroxy-, 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, Glycerin, 1,3-Propanediamine, 1,2-Propanediol 2-acetate, Butane, 1-(ethenyloxy)-3-methyl-, Propane, 1,1-diethoxy-,1H-Imidazole-4-carboxamide, 5-amino-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Resorcinol, Phenol, 2,6-dimethoxy-,6H-Purin-6-one, 2-amino-1,7-dihydro-,6H-Purin-6-one, 2-amino-1,7-dihydro-, 1,4-Benzenediol, 2-methoxy-, Phenol, 3,4-dimethoxy-, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, Phenol, 4-[2-(dimethylamino)ethyl]-, 1-Butanol, 2-amino-, 3-Hydroxy-4-methoxybenzoic acid, Phenol, 3,4,5-trimethoxy-, Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-, 7H-Furo[3,2-g] benzopyran-7-one, n-Hexadecanoic acid, Pentanoic acid, 10-undecenyl ester, Octadecanoic acid [36].

Microscopical studies

A thin transverse section of the fresh root of *Borassus flabellifer* was taken using microtome and studied. Phuloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope [37]. The outermost layer was found to be the rhizodermis. It was made up of thin-walled, rectangular parenchymatous cells which were arranged compactly without intercellular spaces. Exodermis was found beneath the epidermis and composed of two to three layers of sclerified parenchyma. The cortex was wide, extensive and made up of several rows of thin walled parenchyma showing intercellular spaces. Beneath this layer aerenchyma cells with large intercellular space was found [38]. The cells found were non-lignified in this region. Stele showed three specific regions: pericycle, vascular bundles, and medulla or pith. Vascular bundles contained 18-20 pairs of radial polyarch xylem and phloem cells. Medulla or pith was found as wide central part of the stele. It was made up of thin-walled parenchyma cells.

Sclerified parenchyma was found scattered in the powder. Xylem vessels were found to be lignified, pitted walls and with spiral arrangements [39]. Fibers of phloem were found to be lignified with a lumen in it. Calcium oxalates were present in abundance and were of prismatic and rectangular in shape. Starch grains present were circular to oval in shape. Polygonally shaped parenchyma cells were found throughout the powder.

Determination of physicochemical properties

Total ash, acid insoluble ash and water soluble ash of *Borassus flabellifer* root was determined by standard methods. The crude fibre content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and petroleum ether soluble extractive values [40] were evaluated. Fluorescence analysis under ultraviolet light after treatment with various chemical and organic reagents was determined [41, 42].

Pharmacological actions

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using acute and chronic models like; carrageenan-induced paw oedema like cotton pellet induced granuloma and carrageenan-induced air-pouch model in rats for the ethanol extract of male flowers. The animals were divided into four groups (n = 6). Group I served as Control received the vehicle only (1% Carboxymethylcellulose, CMC, 10 ml/kg p. o.). Group II served as Standard, received Diclofenac Sodium at a dose of 100 mg/kg b.w. Group III and IV served as a test, received ethanolic extract at doses of 150 and 300 mg/kg b.w. p. o. respectively [43].

Nystatin-induced rat paw edema model was employed to investigate the anti-inflammatory activity of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L (Arecaceae). The extract at doses 200 mg/kg b.w. and 400 mg/kg b.w. and diclofenac sodium (standard) at 100 mg/kg b.w. showed significant anti-inflammatory when compared to control ($p<0.0001$) [44].

Antiarthritic activity

The ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L (Arecaceae), was screened for anti-arthritic potential of the extract by Freund's Complete Adjuvant (FCA) induced polyarthritis. Rats were injected, 0.1 ml of FCA into the subplantar region of the right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer. All the animals received either extract or diclofenac sodium or vehicle (1% CMC) orally depending upon their respective grouping for 21 consecutive days from the day of FCA injection. On 21st day, rats were anaesthetized using diethyl ether and oedematous tissues were isolated from the injected hind paw and were assayed for hydroxyproline [45], hexosamine [46, 47] and total protein content [48]. Blood was withdrawn from retro-orbital plexus of all the groups, and various haematological, and biochemical parameters were estimated.

Cytotoxic activity

The seed coat of *Borassus flabellifer* extracts was tested for inhibitory effect on HeLa Cell Line. The cytotoxicity of *Borassus flabellifer* on HeLa cell was evaluated by the MTT assay. In concentration range between 32 µg/ml to 750 µg/ml. *Borassus flabellifer* were administered at different concentrations viz., 32, 64, 128, 256, 500 and 750 µg/ml and found that the growth of the HeLa cells was significantly inhibited [49].

Antibacterial activity

The antibacterial activity of methanol extract of *Borassus flabellifer* L. (Arecaceae) seed coat (soft outer shell) was studied by agar well diffusion method *in vitro*. The antibacterial potential was examined against Gram-positive bacteria, i.e., *Staphylococcus aureus, Bacillus subtilis and G*ram-negative bacteria, i.e., *Klebsiella pneumonia* and *Serratia marcescens* [50]. The methanol extract of the seed coat has showed consistently significant inhibitory activity on different bacterial species tested. Furthermore, the minimum inhibitory concentration studies carried out by broth dilution assay and found the MIC ranged between 100 µg to 1 mg/ml implying the significance of antibacterial activity of *Borassus flabellifer*.

Analgesic activity

The ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L.(Arecaceae) were investigated at doses 150 mg/kg b.w. and 300 mg/kg b.w. using acetic acid induced writhing [51], hotplate [52], tail-clip [53] method. Oral administration of *Borassus flabellifer* ethanolic extract (BFEE) produced significant ($P<0.0001$) reduction in no. of writhes induced by acetic acid. Moreover, in the hot-plate test, (BFEE) significantly ($P<0.0001$) raised the pain threshold at the different time of observation (0-60 min) in comparison with control. In tail-clip test also, the extract caused a significant ($P<0.0001$) inhibition of pain at both the doses used. There was a significant dose-dependent inhibition of both phases of the formalin-induced pain response in mice.

Antipyretic activity

Antipyretic activity was measured by slightly modifying the method described by Adams *et al* [54]. The ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L.(Arecaceae) was tested for antipyretic activity. There was a significant dose-dependent inhibition of both phases of the formalin-induced pain response in mice. Tested on yeast-induced pyrexia in rats, BFEE significantly ($P<0.0001$) reversed hyperthermia at either dose.

Hypoglycemic activity

The hypoglycemic effects were investigated in the alcoholic extract of *B. flabellifer* in normal and diabetic rats. Diabetes was induced in rats by single dose administration of alloxan (120 mg/kg, i. p.) or by injecting dexamethasone (10 mg/kg, i. p.) for 10 d. In normal rats, (100, 200 and 400 mg/kg) had significantly decreased the blood glucose level in a dose-dependent manner after repeated administration for 7 d. In alloxan induced diabetic rats, extract had decreased blood sugar level and improved glucose tolerance in alloxan induced diabetic rats at the end of 1st, 2nd, 3rd and 4th week after extract test treatment. However, the insulin levels of extract treated group did not significantly change after 28 d treatment with the extract.

Antioxidant activity

The fruits of *Borassus flabellifer* Linn. belonging to family Areaceae reported to be possess the antioxidant activity. The extracts have also been evaluated for antioxidant activity by using *in vitro* methods DPPH (2, 2-Diphenyl-1 picrylhydrazyl) and ABTS (2, 2-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) assay. The effect of the plant extract of the DPPH was estimated according to the method of *Hou et al.* with minor modification. ABTS diammonium salt radical cation decolorization test was performed using the spectrophotometric method of pellegrini *et al.* [55]. The root of *B. flabelifera* was investigated for antioxidant activity by the phospho-molybdenum method according to the procedure of Prieto *et al.,*. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature [56]. The free radical scavenging capacity of the extracts was determined using DPPH Serial dilutions were made and the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a standard. The inhibition curve was plotted, and IC_{50} values were calculated [57, 58]. The reducing power was determined according to the method previously described by Oyaizu. The absorbance was measured at 700 nm. Ascorbic acid was used as a reference standard. Phosphate buffer (pH) was used as a blank solution [59]. The total antioxidant activity of ABTS radical scavenging assay the three extracts was evaluated according to the decolorization of the ABTS radical cation (ABTS+) as percentage inhibition by Re *et al.* The absorbance was measured at 734 nm [60]. The ability of the extract to reduce ferric ions was determined using the FRAP assay developed by *Benzie and Strain* [61] (1996). Appropriate dilutions of extracts were prepared, and 100 µl was mixed to 900 µl of FRAP reagent, vortexed and incubated at 37 0C for 4 min. The absorbance was measured at 593 nm and reported as BHT equivalents (µg 100 mg-1 EY).

Determination of the MIC of the methanol extracts by broth dilution assay

The medium containing different concentrations of methanol extract of seed coat of *Borassus flabellifer* viz., 10, 1, 0.1, 0.01, 0.001 mg/ml prepared by serial dilution. After inoculation, the tubes were incubated for 24 h at 37 °C. The MIC of each sample was determined by measuring the optical density in the spectrophotometer at 620 nm and compared the result with those of the non-inoculated broth [62].

CONCLUSION

Borassus flabellifer is a medicinal plant with innumerable medicinal qualities for all parts used since ancient times. Besides the plant having traditional uses it is also used for people who make their living from this tree using its wood, fruits, sap, stems, petioles and leaves to process a variety of food products, beverages, furniture, building materials, and handicrafts. In this review, an attempt was made to provide traditional uses and pharmacological aspects of *Borassus flabellifer*, a medicinal plant native to Southeast Asia. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind.

ACKNOWLEDGMENT

The authors were thankful to A. U College of Pharmaceutical Sciences, Andhra University for providing constant encouragement and support and for all the necessary laboratory facilities throughout our work.

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Brahman M. Indigenous medicinal plants for modern drug development programme: revitalization of native health tradition. Adv Plant Sci 2000;1391:1-10.
2. Pushapangadan P, Iyengar PK, Damodaran VK. Role of traditional medicine in primary health care. Science Health; 1995.
3. Chu C, Xia L, Bal LP, Li P, Chen HB, Zhao ZZ. Authentication of 31 species of toxic and potent Chinese material medica by light microscopy, Part 3: two species of T/PCMM from flowers and their common adulterants. Microsc Res Tech 2009;72:454-63.
4. Joshi CS, Ekambaram Sanmuga Priya, Subramaniam Venkataraman. Acute and subacute toxicity studies on the polyherbal ant diabetic formulation diakyur in experimental animal models. J Health Sci 2007;53:245-9.
5. Rajeshwari Sivaraj, Balakrishnan A, Thenmozhi M, Venkatesh R. Preliminary phytochemical screening of *Aegle marmelos*, *Ruta graveolens*, *Opuntia dellini*, *Euphorbia royleana* and *Euphorbia antiquorum*. Int J Pharm Sci Res 2011;2:146-50.
6. http://plants.usda.gov/core/profile?symbol=BOFL2. [Last accessed on 10 Jan 2015].
7. http://www.thesaurus.net/borassus-flabellifer. [Last accessed on 10 Jan 2015].
8. http://www.flowersofindia.net/catalog/slides/Palmyra%20Palm.html. [Last accessed on 10 Jan 2015].
9. http://eol.org/pages/1123573/names/common_names. [Last accessed on 10 Jan 2015].
10. Nesbitt M. The cultural history of plants. Taylor Francis; 2005. p. 173.
11. Nadkarni, Indian Materia Medica, (Popular Prakashan, Bombay); 2002. p. 209-10.
12. Paschapur MS, Patil S, Patil SR, Kumar R, Patil MB. Evaluation of the analgesic and antipyretic activities of ethanolic extract of male flower (inflorescences) of *Borassus flabellifer* L. (Arecaceae). Int J Pharm Pharm Sci 2009;1:98-106.
13. Paschapur MS, Patil MB, Kumar R, Patil SR. Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) in experimental animals. J Med Plants Res 2009;3:49.
14. Paschapur MS, Patil MB, Kumar R, Patil SR. Influence of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) on chemically induced acute inflammation and polyarthritis in rats. Int J PharmTech Res 2000;1:551.
15. Révész L, Hiestand P, LaVecchia L, Naef R, Naegeli HU, Oberer L, *et al.* Isolation and synthesis of a novel immunosuppressive 17alpha-substituted dammarane from the flour of the Palmyrah palm (*Borassus flabellifer*). Bioorg Med Chem Lett 1999;9:1521-6.
16. Devi S, Arseculeratne SN, Pathmanathan R, McKenzie IF, Pang Tm. Suppression of cell-mediated immunity following oral feeding of mice with palmyrah (*Borassus flabellifer L*) flour. Aust J Exp Biol Med Sci 1985;63:371.
17. Andersen PH, Poulsen E. Mutagenicity of flour from the palmyrah palm (*Borassus flabellifer*) in *Salmonella typhimurium* and *Escherichia coli.* Cancer Lett 1985;26:113-9.
18. Kangwanpong D, Arseculeratne SN, Sirisinha S. Clastogenic effect of aqueous extracts of palmyrah (*Borassus flabellifer*) flour on human blood lymphocytes. Mutat Res 1981;89:63-8.
19. Sumudunie KA, Jansz ER, Jayasekera S, Wickramasinghe SM. The neurotoxic effect of palmyrah (*Borassus flabellifer*) flour re-visited. Int J Food Sci Nutr 2004;55:607-14.

20. Van Rheedede's Hortus malabarus. Vol. ed. English; 2013. p. I 23-4.
21. Masayuki Yoshikawa, Fengming Xu. New spirostane-type steroid saponins with antidiabetogenic activity from *Borassus flabellifer*. Chem Pharm Bull 2007;55:308-16.
22. Kapoor LD. Hand Book of Ayurvedic Medicinal Plants. CRC Press: Newyork; 2005. p. 82.
23. Nadkarni KM. Indian materia medica. Vol. 1. (Popular Prakashan, Bombay); 2002. p. 209-10.
24. Morton JF. Notes on distribution, propagation and products of *Borassus* palms (Arecaceae). Econ Bot 1988;42:420-41.
25. Pandey MM, Rastogi S, Rawat AK. The International Journal of Alternative Medicine 2008;2:5-9.
26. Pattanaik C, Reddy CS, Dhal NK. Phytomedicinal study of coastal sand dune species of Orissa. Indian J Traditional Knowledge 2008;7:263-8.
27. Masayuki Y, Fengming X, Toshio M, Yutana P, Seikou N, Yasunobu A, *et al*. Medicinal flowers. XII.(1)) New spirostane-type steroid saponins with antidiabetogenic activity from Borassus flabellifer. Chem Pharm Bull 2007;55:308-16.
28. Sudhakara P, Kannan P, Obireddy K, Varada Rajulu. Organophosphorus and DGEBA resins containing clay nanocomposites: flame retardant, thermal, and mechanical properties. J Mater Sci 2011;46:5176.
29. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 2nd ed. (London: Chapman and Hull); 1991.
30. Sofowora A. Medicinal Plants and Traditional Medicines in Africa, (Chichester John Wiley and Sons New York; 1933. p. 97-145.
31. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plants Res 2009;3:67-72.
32. Pullaiah T. Flora of Andhra Pradesh (India). Vol. III. Scientific Publishers, Jodhpur; 1997. p. 1015.
33. Nadkarni KM, Indian materia medica. Vol. 1. (Popular Prakashan, Bombay); 2002. p. 209-10.
34. Morton JF. Notes on distribution, propagation and products of borassus palms (Arecaceae). Econ Bot 1988;42:420-41.
35. Masayuki Y, Fengming Xu. New spirostane-type steroid saponins with antidiabetogenic activity from *Borassus flabellifer*. Chem Pharm Bull 2007;55:308-16.
36. Subashini S, Ramesh Kannan V, Mani P. Phytochemical and GC-MS analysis of bioactive compounds from *Borassus flabellifer* Linn root. Eur J Mol Biol Biochem 2015;2:148-52.
37. Khandelwal KR. Practical pharmacognosy techniques and experiments. 9th ed. (Nirali Prakashan, Pune); 2002. p. 146-60.
38. Kailasnath Sarma T, Rama Krishna KN, Sai Siva Ramakrishna V, Gourinath A, Satyanarayana Reddy P. Intermediate first year botany, *Telugu Akademi Publication*, Hyderabad; 2004. p. 220-2.
39. Luiz Alfredo Rodrigues Pereira, Maria Elisa Ribeiro Calbo, Claiton Juvenir Ferreira. Anatomy of pneumatophore of mauritia vinifera mart. Braz Arch Biol Technol 2000;43:327-33.
40. Ansari SH. Essentials of pharmacognosy. 4th ed. (Birla Publications, Delhi); 2010. p. 589-93.
41. Madhavan V, Hema Basnett, Guru Deva MR, Yoganarsimhan SN. Pharmacognostical evaluation of *Drosera burmannii Vahl* (Droseraceae). Indian J Traditional Knowledge 2009;8:326-33.
42. Madhavan V, Pravin kumar P, Zamabad MR, Guru Deva, Yoganarsimhan SN. Pharmacognostical evaluation of root bark of *Streblus asper Lour*. Indian J Traditional Knowledge 2009;8:176-80.
43. Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. Evidence of peroxynitrite involvement in the carrageenan induced rat paw edema. Eur J Pharmacol 1996;303:217-24.
44. Turner RA, Harborn P. Screening methods in pharmacology. Vol. 1. (New York: Academic Press Inc); 1971. p. 302-4.
45. Woessner JR. The determination of hydroxyproline in tissue and protein samples containg small roportions of this amino acid. Arch Biochem Biophys 1961;93:440.
46. Elson LA, Morgan WTJ. Water, electrolyte and nitrogen content of human skin. Proc Soc Exp Biol Med 1933;58:97-100.
47. Davidson E. Analysis of sugars found in mucopolysaccharides. In: Eds. Neufeld EF, Ginsburg V. Methods in enzymology. Vol. 8. New York: Academic press; 1996. p. 52-65.
48. Kind PRN, Ming AJ. Estimation of plasma phosphate by hydrolyzed phenol with amino antipyrine. J Clin Pathol 1954;7:322-7.
49. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
50. Kaladhar DSVGK, Siva Kishore N. Antimicrobial studies, biochemical and image analysis in *Mirabilis jalapa.* Int J Pharm Technol 2010;2:683-93.
51. Koster R, Anderson M, De Beer EJ, Acetic acid analgesic screening. Fed Proc 1959;18:418–20.
52. Franzotti EM, Santos CVF, Rodrigues HMSL. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia L.* (Malva-branca). J Ethnopharmacol 2000;72:273-7.
53. Bianchi C, Franceschini J. Experimental observation on Haffner's method for testing analgesic drugs. Br J Pharmacol 1954;9:280–4.
54. Adams SS, Hebborn P, Nicholson JS. Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent. J Pharm Pharmacol 1968;20:305–12.
55. Ghoghari AM, Bagul MS, Anandjiwala S, Chauhan MG, Rajani M. Free radical scavenging activity of *Aspidium cicutarium* rhizome. J Nat Rem 2006;6:131-4.
56. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phospho-molybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
57. Hasan MS, Ahmed MI, Mondal S, Uddin SJ, Masud MM, Sadhu SK, *et al*. Antioxidant, antinociceptive activity and general toxicity study of *Dendrophthoe falcata* and isolation of quercetin as the major component. Orient Pharm Exp Med 2006;6:355-60.
58. Alam MA, Nyeem MAB, Awal MA, Mostofa M, Alam MS, Subhan N, *et al*. Antioxidant and hepatoprotective action of the crude methanolic extract of the flowering top of *Rosa damascena*. Orient Pharm Exp Med 2008;8:164-70.
59. Oyaizu M. Studies on the product of browning reaction prepared from glucose amine. Jpn J Nutr 1986;44:307-15.
60. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 1999;26:1231-17.
61. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70-6.
62. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48:5-16.