Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
www.plantsjournal.com
JMPS 2020; 8(4): 01-05

Received: 01-05-2020
Accepted: 03-06-2020

Aishwarya Avinash Shinde
Amrutvahini College of Pharmacy Sangamner Maharashtra, India

Dr. DP Hase
Amrutvahini College of Pharmacy Sangamner Maharashtra, India

Corresponding Author:
Aishwarya Avinash Shinde
Amrutvahini College of Pharmacy Sangamner Maharashtra, India

Isolation of carpaine from *Carica Papaya* leaves by using LCMS

Aishwarya Avinash Shinde and Dr. DP Hase

Abstract
Papaya (*Carica papaya* Linn.) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. Since, each part of papaya tree possesses economic value, it is grown on commercial scale. During the last few decades considerable progress has been achieved regarding the biological activity and medicinal application of papaya and now it is considered as valuable nutraceutical fruit plant. Carica papaya, is an lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties.

Keywords: Carica Papaya, LCMS

Introduction
Herbal medicine (Herbalism) is the study of botany and the use of medicinal plants. Plants have been the basis of medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based drugs. Although herbalism may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, few high-quality clinical trials and standards for purity or dosage exits. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts.
Herbal Medicine is also called Phytomedicine or Phototherapy. Paraherbalism describes alternative and pseudoscientific practices of using unrefined plant or animal extract as unproven medicines or health-promoting agents. Para herbalism differs from plant-derived medicines is standard pharmacology because it does not isolate or standardize biologically active compounds, but rather relies on the belief that preserving various substances from a given source with less processing is safer or more effective for which there is no evidence. Herbal dietary supplements most often fall under the Phytotherapy category.

Aim and Objectives
Phytochemical analysis is intended to screen, identify, extract and isolate the phytoconstituents to evaluate the therapeutic potential of plant and to develop phytochemical standards for medicinal plant material for the quality control purpose. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive products.
It also provides with chemical fingerprint of said herbal extract. This fingerprinting is done by qualitative analysis and further can be validated by quantitative assay too.
LCMS has been also used to analyses and quantify drugs and plant metabolites contained in biological samples.
To explain the basic principle of LCMS/MS to describe the general characteristics of analytical LCMS/MS applications and to comprehensively discuss the application of technology in the field.

Carica Papaya

The papaya is a small, sparsely branches tree, usually with a single stem growing from 5 to 10 m (16 to 33ft) tall, with spirally arranged leaves. Confined to the top of the trunk. The lower trunk conspicuously scarred where leaves and fruit were borne. The leaves are large, 50-70 cm (20-28 in) in diameter, deeply palmately lobed, with seven lobes. All part of the plants contain latex in articulated laticifers. Papayas are dioecious. The flowers are 5- parted and highly dimorphic, the male flowers with the stamens fused to the petals. The female flowers have a superior ovary and five contorted petals loosely connected at the base.

Fig 1: *Carica papaya* tree, flower, and ripe fruit.

Table 1: Botanical Classification

Domain:	Flowering plant
Kingdom:	Plantae
Sub Kingdom:	Tracheobionta
Class	Magnoliopsida
Subclass:	Dilleniidae
Super division:	Spermatophyta
Phylum	Steptophyta
Order:	Brassicales
Family:	Caricaceae
Genus:	*Carica*

Botanical Name: *Carica papaya* Linn

Chemical Constituents of Carica papaya

Carica papaya Linn is one of the valuable plant used for various purposes in medicinal field. Leaves, fruit and seeds of the Carica papaya are used as ethno medicine this work describes biochemical constituents of leaves of Carica papaya. Chemical composition of various part of Carica papaya plant are describe.

Table 2: Chemical composition of various parts of Carica papaya Plant.

Part	Constituents
1. Fruit	protein, fat, fibre, carbohydrates, minerals, calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, niacin, and caroxene, amino acid, citric acids and molic acid (green fruits), volatile compounds : linalol, benzylisothiocynate, cis and trans 2, 6-dimethyl-3,6 expoxy-7 octen-2-ol. Alkaloid, α; carpaine, benzyl-β-d glucoside, 2-phenylethl-β-D-glucoside, 4-hydroxyl -phenyl-2
2. Juice	N-butyric, n-hexanoic and n-octanoic acids, lipids; myristic, palmitic, stearic, linoleic, linolenic acids-vaccenic acid and oleic acids
3. Seed	Fatty acids, crude proteins, crude fibre, papaya oil, carpaine, benzylisothiocynate, benzylglucosinolate, glucotropacolin, benzylthiourea, hentriacontane, β-sistosterol,
	caricin and an enzyme nyrosin
4. Root	Arposide and an enzyme myrosin
5. Leaves	Alkaloids carpain, pseudocarpain and dehydrocarpaine I and II, choline, carposide, vitamin C and E
6. Bark	β-sitosterol, glucose, fructose, sucrose, galactose and xylitol
7. Latex	proteolytic enzymes, papain and chemopapain, glutamine cyclotransferase, chymopapain A, B and C, peptidase A and B and lysozymes

Plant material and authentication

Carica Papaya, Family Caricaceae matured healthy leaves of male and female of from Sangamner, Maharashtra, India in September 2019. Mature leaves of male and female plant of *Carica Papaya*were thoroughly washed with distilled water to remove dust. Petiole and veins were removed. Remaining portion was shade dried for a day followed by complete drying in an oven at 38 C, grounded using a mortar and pestle for 10-15 min and the powder was preserved.

Solvents and reagents

Analytical grade Methanol (Sigma Aldrich), Acetonitrile (Thomus Baker) were used for LC-MS analysis. Anisaldehyde (Sigma Aldrich) were used for derivatising reagent, formic acid & water etc.

Extraction procedure

Preparation of leaf extract

Healthy, fresh young leaves of *Carica papaya* were collected from Sangamner, Maharashtra, India in September 2019. The leaf extract was prepared with slight modification. Leaves were rinsed under tap water and then by double distilled water then dried at room temperature for 15 days. 20g dried leaves were grounded to powder using a mixer grinder. 20g dried leaves powder was soaked in chloroform for extraction in a rotary shaker for 3-5 days. Dried leaves of *C. papaya* L. were ground in a cross beater mill equipped with a 1 mm sieve. The *C. papaya* leaves were extracted and extract partitioned into fractions using slightly modified methods as follows: 500 g of dark brown, smooth textured powdered sample was extracted with 1.5 l of petroleum ether using a Soxhlet extractor for six hours. The petroleum ether extract obtained was discarded. The marc was further extracted with 1.5 l of aqueous methanol (1:3 v/v) using the soxhlet extractor for another six hours. The obtained methanolic extract was then evaporated to dryness using vacuum rotary evaporator.

The methanolic extract was then dissolved in 10% sulfuric acid solution and partitioned with hexane, chloroform, ethyl acetate and n-butanol successively to give hexane fraction - 23.9 g, chloroform fraction- 5.23 g, ethyl acetate fraction - 2.68 g, n-BuOH fraction 5.50 g and remaining aqueous fractions - 34.2 g. All extracts and fractions were encoded as: CE – crude methanol extract; HF- hexane fraction; CF- chloroform fraction; EF-ethyl acetate fraction; BF-nbutanol fraction; AF- aqueous fraction remaining after fractionation. All extract fractions were freeze dried and stored at 4°C.

LC-MS (Liquid chromatography- mass spectrometry)

Liquid chromatography–mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds

commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring, food processing, and pharmaceutical, agrochemical, and cosmetic industries.

In addition to the liquid chromatography and mass spectrometry devices, an LC-MS system contains an interface that efficiently transfers the separated components from the LC column into the MS ion source [2, 3]. The interface is necessary because the LC and MS devices are fundamentally incompatible. While the mobile phase in a LC system is a pressurized liquid, the MS analyzers commonly operate under high vacuum (around 10−6 torr / 10−7 "Hg). Thus, it is not possible to directly pump the eluate from the LC column into the MS source. Overall, the interface is a mechanically simple part of the LC-MS system that transfers the maximum amount of analyte, removes a significant portion of the mobile phase used in LC and preserves the chemical identity of the chromatography products (chemically inert). As a requirement, the interface should not interfere with the ionizing efficiency and vacuum conditions of the MS system [2]. Nowadays, most extensively applied LC-MS interfaces are based on atmospheric pressure ionization (API) strategies like electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo-ionization (APPI).These interfaces became available in the 1990s after a two decade long research and development process [4].

Table 3.

Acronym	LCMS
Classification	Chromatography Mass spectrometry
Analytes	organic molecules bimolecular
Manufacturers	Agilent
	Bruker
	PerkinElmer
	SCIEX
	Shimadzu Scientific
	Thermo Fisher Scientific

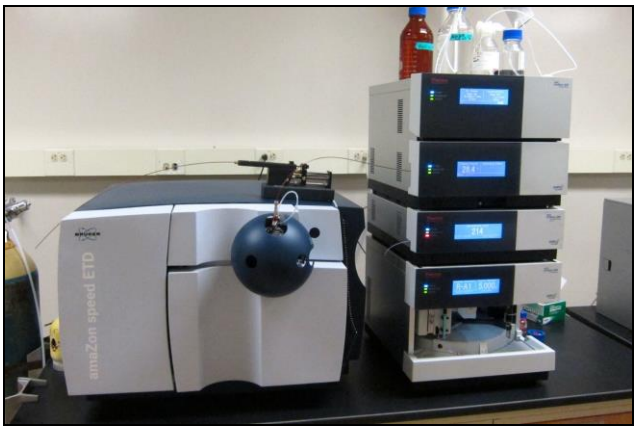

Fig 2: Liquid chromatography- mass spectrometry

Result and Conclusion

Study of Morphological Characters of Plant *Carica papaya leaves.*

Table 3.

S. No	**Parameter**	**Morphological Character**
1	Color	Green
2	Shape	Star shaped
3	Height	50-70 cm diameter
4	Odour	Aromatic

Powder characteristics of *Carica papaya* (Lin)

The powder of *Piper nigrum* fruit is Blackish grey, with aromatic odour and pungent in taste. The microscopic examination of the powder shows epicarp, mesocarp, sclereid, yellow colourtesta, beaker shape stone cells, starch and isolated oil cells.

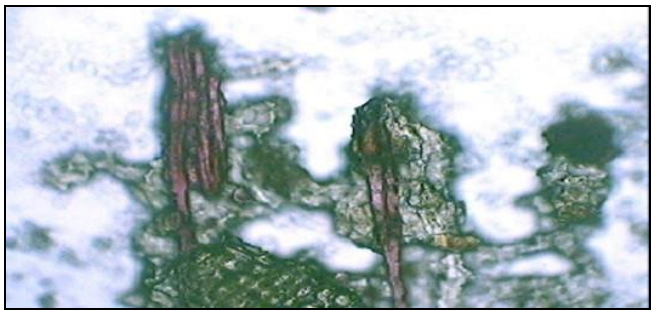

Fig 3:

Papaya (*Carica papaya* L.) contains alkaloid compounds, saponins and flavonoids in its leaves, root, and bark. The phytochemical screening of simplicial powder and plants samples in wet form can identify the presence of alkaloid compounds, flavonoids, steroids, tannins, and saponins [1, 2]. *Carica papaya* L. is one of the plants that has the anticancer ability by increasing apoptosis and inhibiting proliferation of cells. Papaya leaves methanol extract has inhibitory activity against DNA enzyme Topoisomerase II. This is an enzyme that plays an important role in the process of replication, transcription, DNA recombination and proliferation of cancer cells [1, 3]. This study was aimed to observe papaya leaves extract as anticancer through inhibition of cell proliferation and apoptosis induction. It was exploratory research to observe the presence of chemical compounds contained in papaya leaves from LIPI (Indonesian Research Center) Conservation Hall Plant Garden Purwodadi Pasuruan, East Java. This research has ethical innovation established by Research Ethics Commission of Faculty of Veterinary Medicine of Airlangga University with Number: 408-KE.

The papaya leaves which were slightly darker green coloured were taken during sunny weather conditions [2]. Papaya leaves were extracted using methanol solvent and adjusted to 9 pH using 5% NH4OH. The chloroformfraction obtained was then evaporated with rotavapor to obtain a chloroform fraction [2, 4]. The carpaine test was performed using 0.1 g in 10 mL methanol. The sonication was done for 10 minutes at 4500 rpm. The supernatant was filtered with PTFE filter 0.2 microns. The filtrate was inserted in vial bottle and 2 ìl injection sample volume was analyzed by LC-MS / MS.

Carpaine analysis with LC-MS

Carpaine compounds were tested using LC-MS/MS equipment [UHPLC (Thermo Scientific ACCELLA type 1250)]. The column used was Hypersil Gold (50mm x 2.1mm x 1.9 ìm). The mobile phase A comprised 0.1% sulfuric acid diluted in distilled water, phase B consisted of 0.1% formic acid diluted in acetonitrile A linear gradient with a speed of 300 ìl/min with motion phase settings was used as follows: 0-0.6 minutes 10% B, 2.5-4.0 minutes 100% B and 4 minutes 10% B. The LC injection volume was 2 ìL. The columns were set to 30° C and the autosampler compartment was set to 16° C. The operation of MS/MS Triple Q (quadrupole) TSQ QUANTUM ACCESS MAX mass spectrometer from Thermo Finnigan with an ionization ion ESI (Electrospray Ionization) was controlled by TSQ Tune software positive mode.

The quantity determination by the SRM (Selected Reaction Monitoring) method was set to 479 m/z as the precursor ion and at 240 m/z as the product ions. The ionization of ESI were following these conditions: spray voltage 3 kV; evaporation temperature 250 æ% C; capillary temperature, 300 æ%C; nitrogen as a 40 psi sheath gas pressure and Aux gas pressure 10 psi with argon gas. All operating conditions were set using the x-calibur From the analysis of papaya leaves extract, there are carpaine compounds based

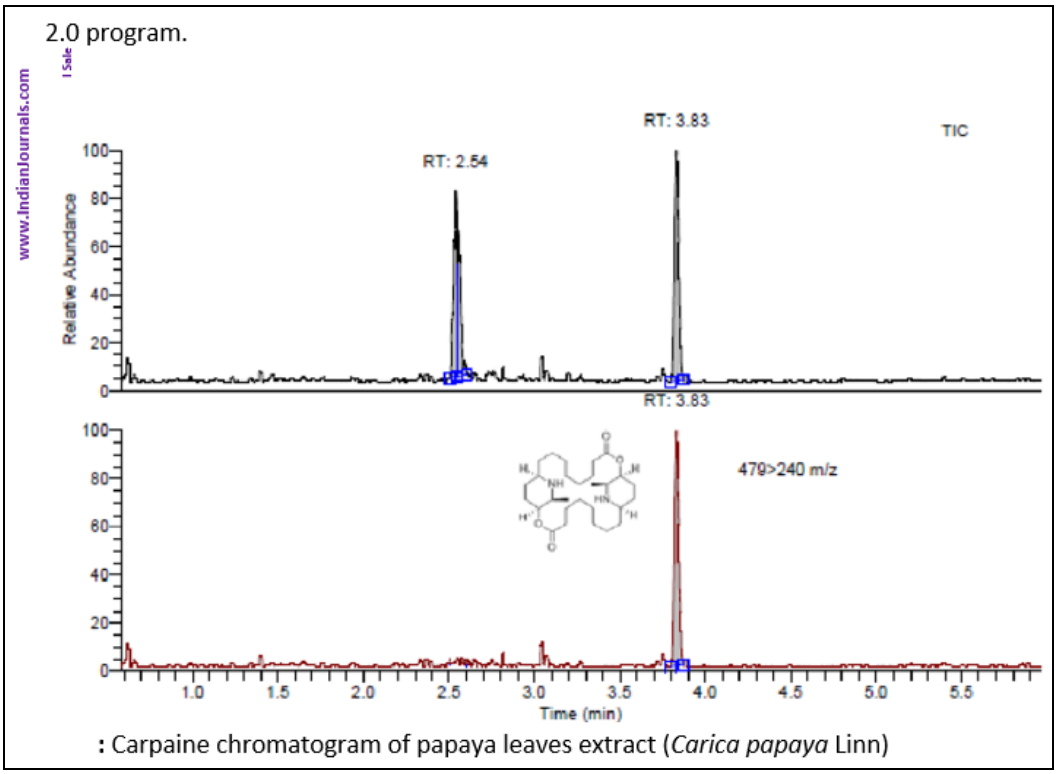

Fig 4.

On LC-MS/MS identification (Fig. 1). There are two chromatograms shown for carpaine identification. There are two peaks on the upper chromatogram as TIC (Total Ion Chromatogram), indicating that there are two compounds that have molecular ions of 479 m/z. In bottom chromatograms as XIC (Extracted Ion Chromatogram), the identification of the carpaine compound shows that there is one peak where the precursor ion is at 479 m/z and the product ion at 240. There is a peak in RT 3.83 minutes suspected as carpaine. The results of this study indicated the identification of alkaloid compound detected in papaya leaves extract. Knowing the potential of alkaloid compounds contained in papaya leaves, they can be used as further study material for the utilization of chemical compounds as drugs [3]. In general, alkaloids are often used in the medical treatment [5]. Carpaine contained in papaya leaves has antitumors, anticancer and antimicrobes properties [2, 5]. Duke [6] reported that the secondary metabolite content of the papaya leaves is, among others, carpaine alkaloids and pseudocarpaineam which are piperidine group of alkaloids. The piperidine-type alkaloid compounds that have anticancer activity and have anticancer mechanism by inducing apoptosis are f lavopiridol compounds which are the resultant compounds of piperidine alkaloids with f lavonoid compounds [7].

References

1. Agrawal DP. Complementary and alternative medicine: An Overview. Curr Sci. 2002; 82:518-524.
2. Dr. Mukherjee PK. Quality control of herbal drugs, 15t edition, business horizon publication, New Delhi, 2002, 247.
3. Kamboj VP. Herbal medicine, CurrSci. 2000; 78:35-39.
4. Verma S, Singh SP. Current & Future Status of Herbal Medicine, veterinary world 1: -2006, 347-350.
5. Tripathi KD. Essentials of Medical Pharmacology, 5th Edn, Jaypee Brothers Medical Publishers Ltd., 2003:453.
6. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology, Elservier, 5th Edn, 2006, 246.
7. Naithani HB, Sahni KC. Forest Flora of Goa. International Books distributors, Deharadun. 1st ed, 1997, 318.
8. Kiritikar KR, Basu BD. Indian Medicinal Plants. Lalit Mohan Basu, Deharadun. 2nd ed, 2003; (1):1251-1252.
9. Umachigi SP *et al*. Antimicrobial, wound healing and antioxidant activity of Anthocephaluscadamba. Afr J of Trad, Compl and Alt Med. 2007; 4(4):481-87.
10. Patel D, Kumar V. Pharmacognostical Studies of *Neolamarckiacadamba* (Roxb.) Bosser leaf. Int J Green Pharmacy. 2008; 2(1):26-27.
11. Mondal S *et al*. Studies on diuretic and laxative activity of bark extract of *Neolamarckiacadamba* (Roxb.) Bosser. DrugInvention Today. 2009; 1(1):78-80.
12. Gunasekharan R, Divyakant A. Antihelmntic activity of leafalcoholic extract of *Neolamarckiacadamba*. Ind J of Nat Prod. 2006; 22(1):11-13.
13. Khandelwal KR. Practical Pharmacognosy, Techniques and experiments. NiraliPrakashan. 6th edn, 2006; (9):149-156.
14. Gupta V, Bansal P, Niazi J, Kaur G. Anti-anxiety Activity of *Citrusparadisi var.star ruby* Extracts. Int J of Pharm Tech Res. 2:1655-1657.
15. Stahl E, TLC. A laboratory handbook, 2nd edition, Springer International. 2005: 241-247, 688-689, 706-707, 863-88.

16. Patil S, Shetty S, Bhide R, Narayanan S. Evaluation of platelet augmentation activity of *Carica papaya* leaf aqueous extract in rats. J Pharmacog Phytochem. 2013; 1:227-32.
17. Pigli RK, Runja C. Medicinal plants used in dengue treatment. Int J Chem Nat Sci. 2014; 2:70-6.
18. Hettige S. Salutary effects of *Carica papaya* leaf extract in dengue fever patients–a pilot study. Sri Lankan Fam Physician. 2008; 29:17-9.
19. Yunita F, Hanani E, Kristianto J. The effect of *Carica papaya* L. Leaves extract capsules on platelets count and hematocrit level in dengue fever patient. Int J Med Aromat Plants. 2012; 2:573-8.
20. Manohar PR. Papaya, dengue fever and ayurveda. Anc Sci Life. 2013; 32:131-3.
21. Senthilvel P, Lavanya P, Kumar KM, Swetha R, Anitha P, Bag S *et al.* Flavonoid from *Carica papaya* inhibits NS2B-NS3 protease and prevents dengue 2 viral assembly. Bioinformation. 2013; 9:889-95.
22. FAOSTAT. Papaya marketing-general information. GFruit Web site, 2012. http://www.itfnet.org/gfruit/Templates%20English/papaya.market.info.htm.
23. Villegas VN. *Carica papaya* L. in Plant Resources of South- East Asia 2: Edible Fruits and Nuts, E. M. W. Verheij and R. E. Coronel, Eds., PROSEA, Wageningen, The Netherlands. 1997; 2:223-225.
24. Nakasone HY, Paull RE. *Tropical Fruits*, CAB International, Walllingford, NY, USA, 1998.
25. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves, *Inflammopharmacology*. 2008; 16(4):168-173.
26. Agung N, Hesty H, Jae SC, Hee-Juhn P. Identification and quantification of flavonoids in *Carica papaya* L. leaf and peroxynitrite-scavenging activity; Asian Pacific Journal of Tropical Biomedicine. 2017; 7(3):208-213.
27. Tasqiah. HPLC- based activity profiling for antiplasmodial compounds in the traditional Indonesian medicinal plant *Carica papaya* L. Journal of Ethnopharmacology. 2014; 155(1):426-434.
28. Bass LN. Seed storage of *Carica papaya* L. HortScience. 1975; 10:232.
29. Baur X, Sauer W, Weiss W. Baking additives as new allergens in baker's asthma. Respiration 1988; 54:70-72.
30. Dillon S, Ramage C, Drew R, Ashmore S. Genetic mapping of a PRSV- P resistance gene in 'highland papaya' based on inheritance of RAF markers. *Euphytica.* 2005a; 145:11-23.
31. Dillon SK, Drew RA, Ramage C. Development of a co-dominant SCAR marker linked to a putative PRSV-P resistance locus in 'wild papaya'. *Acta Horticulturae* 2005b, 694.
32. Dubey A, Nayak S. *Anthocephaluscadamba*: A Review. Pharmacognosy Journal. 2011; 2(18):71-76.
33. Acharyya S, Dash G, Mondal S, Dash S. Studies on glucose lowering efficacy of the *Anthocephaluscadamba* (ROXB) MIQ. Roots. Int. J Pharma. Bio. Sci. 2010; 1(2).
34. Alam MA, Akter R, Subhan N, Rahman MM, Majumder MM, Nahar L *et al*. Antidiarrhoeal property of the hydroethanolic extract of the hydroethanolic extract of the flowering tops of *Anthocephaluscadamba*. Brazilian J Pharmacog. 2008; 18:155-159.
35. Chandrashekar KS, Borthakur A, Prasanna KS. Anti-inflammatory effect of the methanol extract from *Anthocephaluscadamba* stem bark in animal models. Int. J Plant Biology. 2010; 1:30-32.
36. Dolai N, Karmakar I. Evaluation of antitumor activity and *in vivo* antioxidant status of *Anthocephaluscadamba* on Ehrlich ascites carcinoma treated mice. Journal of Ethnopharmacology. 2012, 865-870.
37. Dogra SC. Antimicrobial agents used in ancient India. Indian journal of history of science. 1987; 22:164-169.
38. Gurjar H, Jain S, Nandanwar R, Sahu R. Phytochemical screening of the stem bark of *Anthocephaluscadamba* (ROXB) MIQ. International Journal of Pharmaceutical Science and Research. 2010, 108-115.
39. Kapil A, Koul IB, Suri OP. Antihepatotoxic effects of chlorogenic acid from *Anthocephaluscadamba*. Phytotherapy research. 1995; 9:189-193.
40. Kumar V, Mahdi F, Chander R, Singh R, Mahdi AA, Khanna KA *et al*. Hypolipidemic and antioxidant activity of *Anthocephalisindicus* (kadam) root extract. Indian journal of biochemistry and biophysics. 2010; 47:104-109.
41. Ling-Li Liu, Ying-Tong Di, Qiang Zhang, Xin Fang, Feng Zhu, Dong-Lin *et al*. Aminocadambines A and B, two novel indole alkaloids from *Neolamarckiacadamba*. Tetrahedron lett. 2010; 51:5670-5673.
42. Mondal S, Dash GK, Acharyya A, Sharma HP. Studies on Diuretic and laxative activity of bark extract of *Neolamarckiacadamba (Roxb*.) Bosser. Drug invention today. 2009; 1:78-80.
43. Mali RG, Mehta AA. A review on anthelmintic plants. Natural product radiance. 2008; 7:466-475.
44. Nagakannan P, Basavaraj D, Veeresh P, Boreddy S. Sedative and antiepileptic effect of *anthocephaluscadamba* ROXB.in mice and rats. Indian J pharmacol. 2011; 43(6):699-702.
45. Umachigi SP, Kumar GS, Jayaveera KN, Kumar KDV, Kumar ACK, Dhanpal R. Antimicrobial, wound healing and antioxidant activities of *Anthocephaluscadamba*. African Journal of Traditional, Complementary and Alternative Medicines. 2007; 4:481-487.
46. Nadkarn AK. Indian MateriaMedica, Popular Prakashan Ltd, VoU. 2007; 430:277-278.
47. Lakshminarasmhan P, Kartnikeman S, Prasanna PV, Singh NP. Botanical survey of India, (BSI), Flora of Maharashtra state, Dicotyledones, 2:320.
48. Majumdar A. Home Remedies in Ayurveda, Edn. 1, Amar granth publication, Delhi: 2002, 296-297.
49. Bhandary MJ, Chandrashekar KR, Kaveriappa KM. Medical ethnobotany of thesiddis of Uttara Kannada district Karnataka. J Ethnopharmacol. 1995; 47(3):149-158.
50. Sanjay PU, Kumar GS, Jayaveera KN, Kishore Kumar DV, Ashok Kumar CK *et al*. Antimicrobial, wound healing and antioxidant activities of *Anthocephaluscadamba*. African Journal of Traditional, Complementary and Alternative Medicines. 2007; 4(4):481-487.
51. Gunasekharan R, Divyakant A. Anthelmintic activity of leaf alcoholic extract of *Neolamarckiacadamba* (Roxb.) Bosser. Indian Journal of Natural Products. 2006; 22(1):11-13.