IJBPAS, December, 2015, 4(12): 6509-6524

ISSN: 2277–4998

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

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIOXIDANT, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF BIOACTIVE FRACTION OF LEAVES OF *MYRICA ESCULENTA* BUCH.-HAM ALONG WITH ITS PHARMACOGNOSTIC AND CHROMATOGRAPHIC EVALUATION

SWATI PUNDIR[1*], SUNIL TOMAR[2], NAVNEET UPADHYAY[3], VIKAS SHARMA[1]

[1]Department of Pharmacognosy, School of Pharmaceutical Sciences, Shoolini University, Solan, H.P, India

[2]Department of Pharmacology, School of Pharmaceutical Sciences, Shoolini University, Solan, H.P, India

[3]Department of Quality Assurance, School of Pharmaceutical Sciences, Shoolini University, Solan, H.P, India

***Corresponding Author: Swati Pundir;** Research Scholar, Department of Pharmacognosy, School of Pharmaceutical Sciences, Shoolini University, Solan- 173212, Himachal Pradesh, India; **E-mail: swati.thakur2788@gmail.com; Contact number: +91-9817837689**

ABSTRACT

Present study is an attempt to investigate the antioxidant, anti-inflammatory and analgesic activity of the standardized ethyl acetate fraction of the leaves of *Myrica esculenta* (ME- EtAC) along with exploration of morphological, microscopical and physico-chemical characters of leaves. Standard WHO procedures were adopted for investigation of microscopical and physico-chemical characters. The antioxidant activity was investigated by using DPPH radical scavenging method, anti-inflammatory activity by Carrageenan induced rat paw edema using Albino wistar rats (150-200g) and analgesic activity by using acetic acid-induced writhing and tail immersion test on Swiss albino mice (18-22g). Pharmacognostical study revealed the presence of various features like covering trichomes, anisocytic type of stomata etc. ME- EtAC expressed potent inhibition of DPPH radical scavenging activity (IC 50 of standard recorded

as 18.62 µg/mL and that of fraction is 40.95µg/mL) and was found to be effective in treating inflammation, equivalent decrease in rat paw volume with respect to standard group; it also showed significant analgesic activities in acetic acid-induced writhing test and tail immersion test. The outcome of present study reveals the presence of potential antioxidant, analgesic and anti inflammatory properties of ME- EtAC which could be exploited further for pharmaceutical applications. This study also provides referential information for identification and standardization of *Myrica esculenta* leaf and its extract.

Keywords: *Myrica esculenta*, anti-inflammatory, anti-oxidant, analgesic, Physico- chemical, Pharmacognostic

List of abbreviations: ME-EtAc: Ethyl acetate fraction-*Myrica esculenta*, HPLC-High Performance Liquid chromatography; UV: Ultra violet spectroscopy.

INTRODUCTION

Herbalism is a conventional medicinal practice based on the use of plants and plant extracts for treating various ailments. According to the World Health Organization (WHO), world's 75% of population is using herbs/ medicinal plants for basic healthcare needs and there are reports that supports that lots of pharmaceutical drugs are derived from both nature and ethanobotanical remedies [1,2]. As stated by WHO the macroscopic and microscopic examination of a medicinal plant is the first initiative step towards establishing its identity and purity and should be accomplished before any tests are attempted [3].

M. esculenta (Family: Myricaceae, Synonym: *Myrica nagi*, Common names: Katphala, Kaphal) is an evergreen dioecious plant widely used traditionally by tribes of sub Himalayan region. It is native of China, Japan, Malaya Islands, West Pakistan and Singapore. In India found in states of Arunachal Pradesh, Meghalaya, Nagaland, Manipur, Mizoram, J&K, Uttrakhand, Sikkim and Himachal Pradesh [4-7]. Fruits are edible (sweet in taste), ethno botanically used in anemia, healing ulcers, pectoral, sedative, stomachic, dysentery [7,8], decoction of stem bark used ethno botanically in chronic bronchitis, chronic dysentery [8-10] cough, ear ache [10,11] gonorrhea, piles, in fever, as astringent, carminative, antiseptic, urinary discharges, piles, tumors, a good snuff in headache, collyrium for ophthalmic and other eye diseases [12]. The stem bark is also chewed

to relieve toothache and a lotion prepared from it finds application for washing putrid sores [4]. Few reported activities of *M. esculenta* are anti allergic [13], anthelmentic [14], antioxidant [15, 16], anticonvulsant, analgesic [17], antimicrobial [18], anti-inflammatory [18, 19] mast cell stabilizing [20], anxiolytic [21], ACE inhibitor [22] etc. In spite of numerous medicinal uses of *M. esculenta* which have just traditional reports and no scientific reports, this research is an attempt to evaluate antioxidant, anti-inflammatory and wound healing potential of leaves of *M. esculenta*. Also standardization parameters for its leaves have not been reported. Hence, the present investigation also involves results of morphological, microscopic and physico-chemical characters.

MATERIALS AND METHODS

Chemicals and apparatus

Chemicals and reagents 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, Carrageenan, Carboxy methyl cellulose, Diclofenac, Quercetin, Aluminium chloride, Folin-Ciocalteu Reagent, Gallic acid, Sodium Carbonate (20 % w/v), Acetyl salicylic acid, Ibuprofen, alcohol, chloroform, chloral hydrate solution, Pentazocine, Glacial acetic acid, phloroglucinol, hydrochloric acid, glycerin, sodium hydroxide, petroleum ether, ethyl acetate, ethanol.

Apparatus: Plethysmometer, Compound microscope, stage micrometer, glass slide, cover slip, watch glass, Bunsen burner, Leica DMLS microscope attached with Leitz MPS 32 camera, Digital electronic balance, Grinding mixer, Hot air oven, sonicator, silica crucible, ash less filter paper (Whatman no.44), petridish, UV apparatus, stoppered conical flask, magnetic stirrer, test tubes, soxhlet apparatus, desiccators, distillation apparatus, TLC plates, sprayer, China dish, Digital electronic weighing instrument, chromatographic paper, Beakers.

Collection and authentication of plant material

Fresh leaves of *M. esculenta* were collected in September- October 2014 from the District Solan, Himachal Pradesh. Leaves were then authenticated from the Dr. Y.S Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. The herbarium sheet of the said sample is linked to UHF-Herbarium with field book number 12553.

Processing and solvent extraction

Leaves were washed and cleaned thoroughly and dried under shade for about 15 days. Shade dried leaves were coarsely powdered with the help of grinding machine. The powdered plant material (500 g) was put in to

Soxhlet's extractor (continuous hot percolation process) and extracted with petroleum ether (60-80°C) to remove fats and waxes, further same plant material extracted with ethanol. After exhaustive extraction, the ethanolic extract was concentrated under reduced pressure at 50–55 °C and was adsorbed with silica and subjected to fractionation with various solvents like chloroform, ethyl acetate & n-butanol. For pharmacological studies, a weighed amount of ethyl acetate fraction was suspended in a 1% (w/v) aqueous acacia solution.

Pharmacognostical studies

Pharmacognostical evaluations of fresh drug including histochemical studies were carried out by carrying free hand sections [23, 24]. Powder microscopy of pulverized shade-dried leaf was carried out. Photographs of all the microscopic features were taken using Swift Ives Camera Lucida. Leaf constants and quantitative microscopic methods like stomatal number, vein islet number, vein termination number etc were carried out using Stage and ocular micrometer [24], Physicochemical constants [3], organic analysis, fluorescence studies were carried out [25].

HPLC profile

HPLC profile of the ME-EtAC was developed as described by Kumar et al. [26]. The HPLC profile was carried out on an Agilent 1200 series (USA), Eclips XBD C18 column, 4.6 × 150 mm, 5 μm particle sizes, and the temperature was maintained at 25 °C. Then, 10 μL of sample was injected into the column and eluted with a constant rate of 0.8 mL/min. HPLC grade water with 0.5 % (v/v) glacial acetic acid and acetonitrile (85/15, v/v) were used as mobile phase in 85:15 ratio. The absorbance detector was operated at 345 nm.

In-vitro Antioxidant activity

Antioxidant activity was evaluated by in-vitro-DPPH radical scavenging model [27]. Scavenging activity of diphenyl-2-picrylhydrazyl (DPPH) radicals of ME-EtAC was measured according to the method described by Ayoola et al. Assays were performed in 3 mL reaction mixtures containing 2.0 mL of 0.1 mM DPPH-ethanol solution, 0.1 mL of deionized H_2O (as control) or test plant extracts and 0.9 mL of 50 mM Tris-HCl buffer (pH 7.4), After 30 min of incubation at room temperature, absorbancies of the reaction mixtures at 517 nm were taken. The inhibitory effect of DPPH was calculated according to the following formula:

$$\textbf{Inhibition (\%)} = [(\textbf{Absorbance}_{control} - \textbf{Absorbance}_{sample})/\textbf{Absorbance}_{control}] \times 100$$

IC_{50} represents the level where 50% of the radicals were scavenged by test samples.

Animals

Study was conducted on Male wistar rats (150-200g) and swiss albino mice (18-22g). The ethical committee of the School of Pharmaceutical Sciences, Shoolini University, approved all protocols. The animals were bred and maintained under standard laboratory conditions: temperature (25 ± 2 °C) and photoperiod of 12 h. Commercial pellet diet (Ashirwad Industries, Chandigarh, India) and water were given *ad libitum.* All animals used in experimental work received human care.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan induced rat paw edema [28]. Edema was induced by injecting 0.1 ml of 1.5% (w/v) carrageenan suspension into the sub plantar region of the right hind paw of the rats. All animals (six per group) were given free access to food and water after the sub plantar injections. Control group rats (n = 6) received saline solution (0.9% (w/v), NaCl) (2 ml/kg) and the reference group received 15 mg/kg indomethacin orally. The test groups of rats were treated orally with 100, 200, 400 mg/kg of the ethyl acetate extract 30 min before the carrageenan injection. The paw volume was measured before administering carrageenan ($V0$) and 1.5, 2.5, 3.5, 5.0 and 6.0 h after (Vt). Inflammation was calculated as the increase in volume (ml) of the paw after treatment subtracted of the basal volume. Results were expressed as percentage of inhibition of edema, calculated according to the following formula:

$$\text{Percentage inhibition} = \frac{(Vt - V0)\ \text{control} - (Vt - V0)\ \text{treated}}{(Vt - V0)\ \text{control}} \times 100$$

Analgesic activity

Acetic acid induced writhing

Mice (n=6) of control group were treated orally with vehicle (3 ml/kg) of 0.3% CMC, while those of test groups with ME- EtAC (100 and 200 mg/kg) once a day for 5 consecutive days. Ibuprofen (200 mg/kg, oral) was administrated to mice as a positive control. Writhing was induced in mice by intraperitoneal injection (10 ml/kg) of 0.6% acetic acid after1 h of the oral administration of tested drugs on the 5th day. The number of writhings was counted over a 15 min period [29].

Tail immersion method

Six mice were administered orally with vehicle (3 ml/kg), pentazocine (30 mg/kg), ME- EtAC (100 and 200 mg/kg). The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0 °C. The latency of tail withdrawal was noted as

reaction time. A cut off time of 10 sec was maintained at 55°C to prevent tissue damage. After 30 minutes of treatment the reaction time was measured [30].

RESULTS

HPLC fingerprinting of *ME-EtAC*

The HPLC chromatogram of ME- EtAC is shown in Figure 1. It showed 4 peaks with varying retention times (4.44, 10.53, 11.92 and 21.76) including the presence of myriconol (4.44), myricetrin (10.53), myricadiol (11.92) and myricetin (21.76).

Macroscopic characters

Myrica esculenta is a sub-temperate evergreen dioecious tree 3-15m high with imparipinnate unipinnate compound leaves arranged in alternate manner. Leaves are simple, crowded towards the ends of branches. Plant picture is given in Figure 2 and observed morphologic features in Table 1.

Microscopic characters

Myrica esculenta has dorsiventral leaf structure. Upper epidermis showed presence of non-glandular/ covering, unicellular trichomes. Single row of palisade cells was found below the layer of epidermis. At the midrib region it is absent and is replaced by collenchymas cells. Below the layer of palisade cells presence of vascular strands and spongy parenchyma was noticed. Vascular bundles containing xylem and phloem were present in midrib portion. Trichomes were found to be absent in the lower epidermis. Various microscopic features observed in transverse section of leaf can be seen in Figure 3.

Surface preparation

Both upper and lower epidermis comprises of polygonal cells with straight or slightly arched anticlinal walls. Trichomes are simple, unicellular and with a very thin wall. Each stoma is surrounded by 2-3 subsidiary cells. The leaves have anisocytic type of stomata on both surfaces. Stomatal index of the lower epidermis is more than the upper epidermis. Pictures of minutely observed leaf lamina are represented in Figure 4 and quantitative details are represented in Table 2.

Powder microscopy

The leaf powder is dark green in color with no odor. On microscopical examination, powder of leaves showed the presence of unicellular trichomes, epidermis, xylem, calcium oxalate crystals, starch grains, fibres, stomatas. Images are presented in Figure 5.

Physico-chemical studies

Different physiochemical parameters were determined for the purpose of standardization. Parameters like foreign matter, moisture content, swelling index,

foaming index, ash value and extractive value (with water) were calculated and summarized in Table 3. The powdered leaves and ME-EtAc were subjected to fluorescence analysis and results are given in Table 4, 5. Leaves of *M. esculenta* were also analyzed for the presence of various inorganic elements and calcium, potassium, iron, sodium and magnesium found to be present.

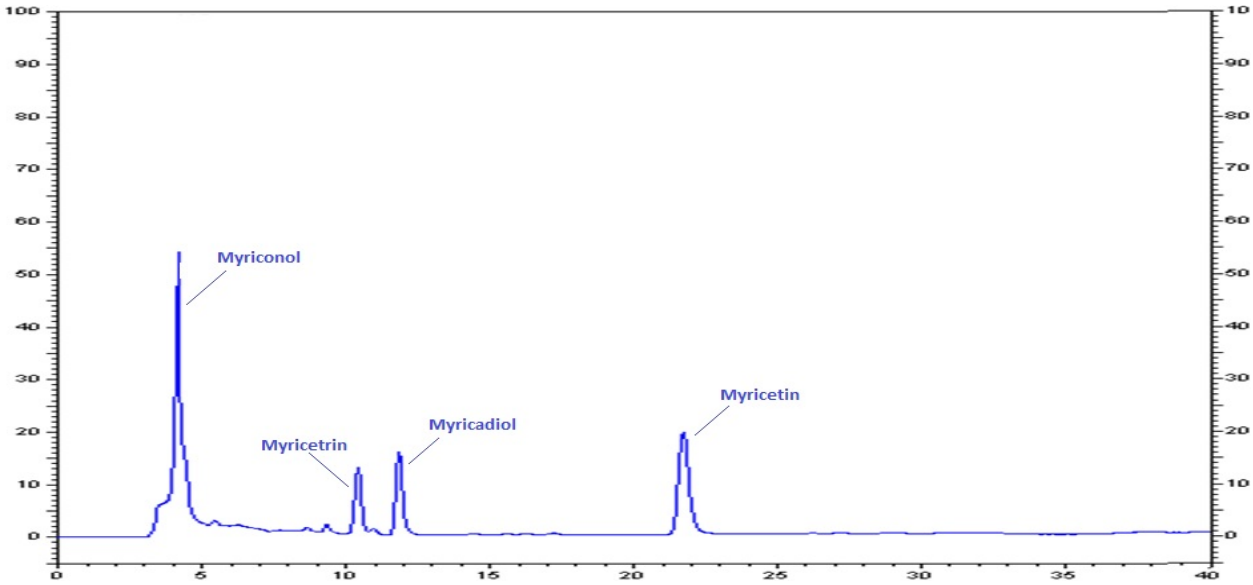
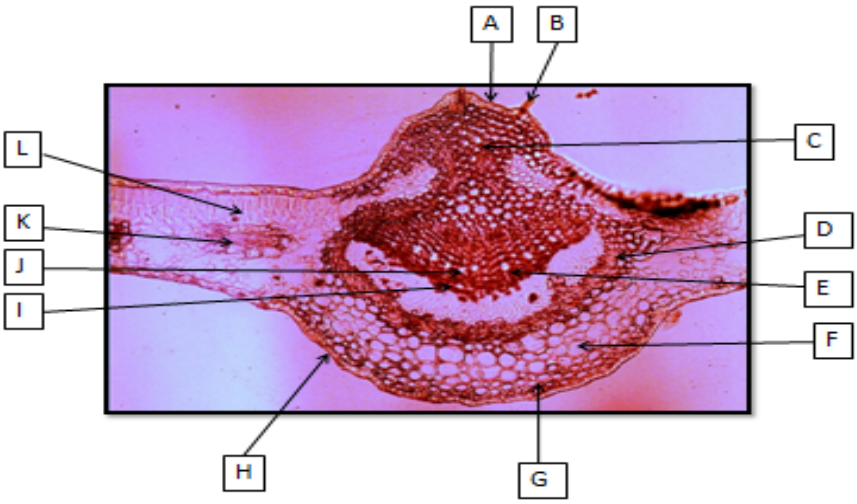


Figure 1: HPLC fingerprinting of ME- EtAC showing presence of myriconol (4.44), myricetrin (10.53), myricadiol (11.92) and myricetin (21.76) in different Rt. The separation was carried out Eclips XBD C18 column, 4.6 ×150 mm, 5 µm particle size, and the temperature was maintained at 25 °C.

Figure 2: Appearance of plant *M. esculenta*

Table 1: Observation of macroscopic features of *M. esculenta*

Sr. No.	FEATURES	OBSERVATIONS
1.	Size	Width= 3.26- 4cm, Length=12-13.5cm
2.	Shape	Lanceolate
3.	Colour	Dark Green
4.	Odour	Odourless
5.	Taste	Tasteless
7.	Apex	Acuminate
8.	Venation	Reticulate
9	Leaf attachment	Petiolate



A-Upper epidermis, B- Covering trichomes, C- Spongy parenchyma, D- Pericycle, E- Xylem, F- Parenchyma, G- Collenchyma, H- Lower epidermis, I- Pericycle, J- Phloem, K- Vascular bundle, L-Palisade cells

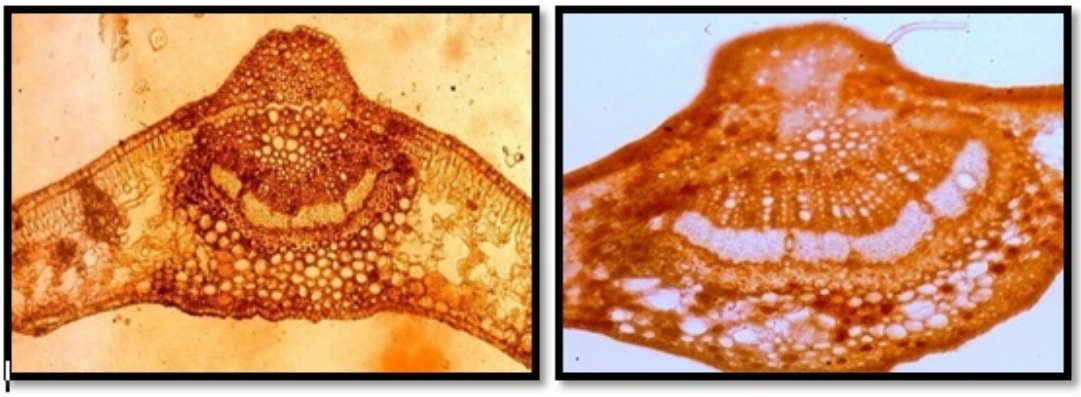


Figure 3: Transverse section showing various characters of *Myrica esculenta*

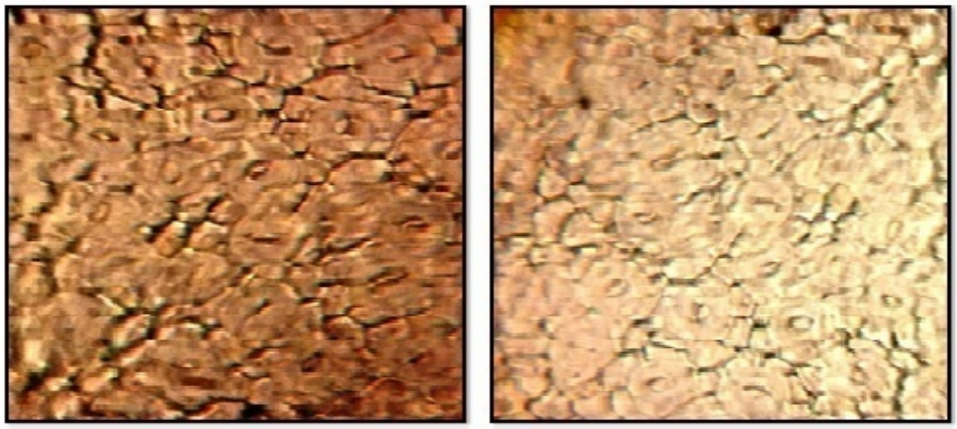


Figure 4: Anisocytic type of stomatas present in leaf of *M. esculenta*

Table 2: Leaf constants of *Myrica esculenta*

Serial No.	Leaf Constants	Value (Range)
1	Stomatal no.	52-56
2	Stomatal index	30.9-31.4
3	Vein islet no.	8-12
4	Vein termination no.	4-8

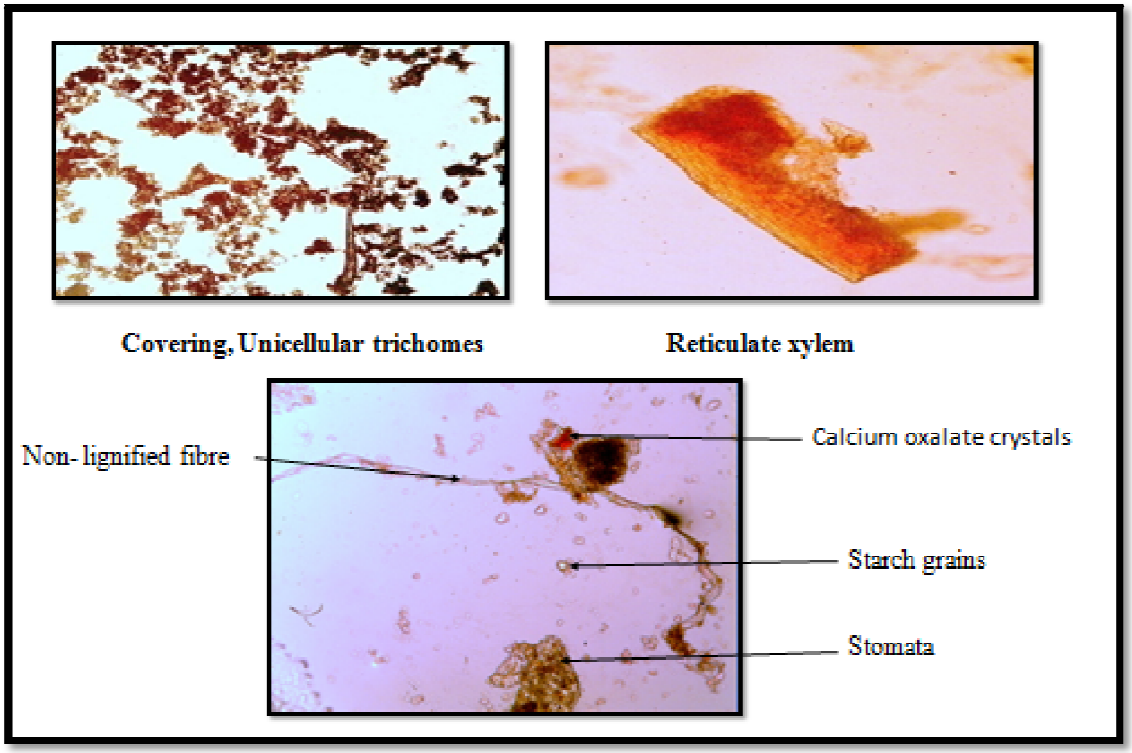


Figure 5: Powder microscopic features of *M. esculenta*

Table3: Results of physicochemical parameters

1	Foreign matter	0.88 % w/w
2	Moisture content	11 % w/w
3	Swelling index	No swelling
4	Foaming index	333.3
5	Ash Value	
	Total ash	0.07mg/g
	Water soluble ash	0.05 mg/g
	Acid insoluble ash	0.02 mg/g
6	Extractive value	
	Cold maceration	2.625 %w/w
	Hot extraction	6.25 %w/w

Table 4: Fluorescence analysis of the powder of *M. esculenta* leaves at day light, UV 254 and 365 nm

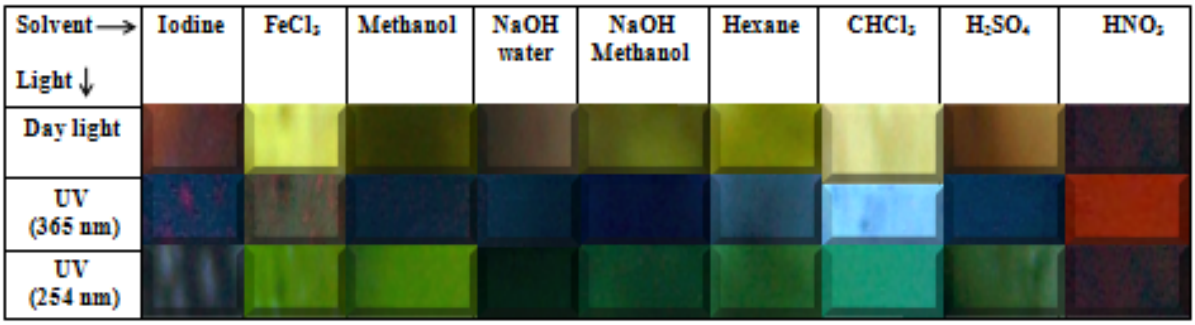
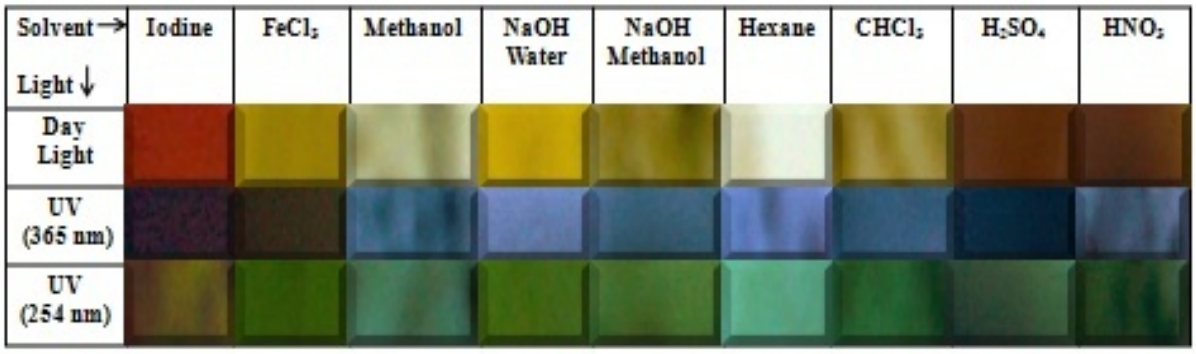


Table 5: Fluorescence analysis of the ME- EtAC at day light, UV 254 and 365 nm



Preliminary phytochemical screening

The results demonstrated the presence of flavonoids, alkaloids, glycosides (anthraquinone), saponins, tannins (condensed), carbohydrates and steroids. Percentage yield of successive extracts with various solvents like petroleum ether, chloroform, ethyl acetate and ethanol were also taken into consideration and which are tabulated in Table 6.

Table 6: Percentage yield of various extracts

Serial no.	Extract	Consistency	Color	Wt. of extract (g)	Percentage yield (% w/w)
1	Petroleum ether	Waxy	Blackish green	4	8
2	Chloroform	Semi solid	Dark green	5.2	10.8
3	Ethyl acetate	Semi solid	Dark green	3.4	7.02
4	Methanol	Solid, Powder form	Blackish green	9.9	23.57

Antioxidant activity

The beneficial effects derived from phenolic compounds have been attributed to antioxidant activity of ME- EtAC. Total phenolic content of plant is recorded as 97.7 and 92.2 µg/mL given in Table 7. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content are given in Table 8. Results for DPPH radical scavenging potential of ME- EtAC is shown through Table 9.

Effect of *ME-EtAC* on anti-inflammatory activity

Anti-inflammatory activity of ME- EtAC against carrageenan induced paw edema shows that the extracts have significant effect on inflammation and markedly reduced the swelling. It shows that the plant extract have significant ($P < 0.01$; $P < 0.001$) anti-inflammatory effect and the results were compared with Diclofenac 10mg/kg and show paw volume reduction. Results are tabulated in Table 10.

Analgesic activity

Effect of *ME-EtAC* on writhing reflex of mice

In the writhing test, intraperitoneal injection of acetic acid evidently resulted in writhing reflexes of mice. ME-EtAC reduced the number of writhing responses, in a dose-dependent manner within 15 min of injection of acetic acid. The most significant response was observed at 200mg/Kg shown in Figure 6.

Effect of *ME-EtAC* on tail immersion in mice

In tail immersion method the ME-EtAC showed dose dependent significant increase in tail withdrawal time from 30 min after administration. ME-EtAC at 200 mg/kg showed significant ($p < 0.001$) analgesic activity after administration. Results are tabulated in Table 11.

Table 7: Total Phenolic content of ME- EtAC Buch.-Ham

ME-EtAC (µg/ml)	Absorbance (at 765nm)	Total Phenolic content (µg/ml)
20	1.891	69.51
40	1.978	72.84
60	2.209	81.7
80	2.482	92.2
100	2.625	97.7

Table 8: Total Flavanoid content of *M. esculenta* Buch.-Ham

ME-EtAC (µg/ml)	Absorbance (at 415nm)	Total flavonoid content (µg/ml)
20	0.494	36.3
40	0.554	42.3
60	0.692	56.1
80	0.754	62.3
100	0.812	68.1

Table 9: DPPH radical scavenging potential of ME- EtAC

Sr No.	Conc. (µg/ml)	Ascorbic acid % scavenging	ME- EtAC % scavenging
1	**0**	0	0
2	**20**	79.78	56.72
3	**40**	84.17	64.09
4	**60**	89.54	67.27
5	**80**	94.32	70.72
6	**100**	100.21	73.18
7	**IC_{50}**	18.62µg/mL	40.95 µg/mL

Table 10: Mean change in paw volume (ml) at different time intervals

Treatment / Dose (mg/Kg)	Mean change in paw vol.(ml) at different time intervals				
	0 h	1 h	2 h	3 h	4 h
Control	0.4±0.036	0.9±0.035	1±0.033	1.2±0.031	0.9±0.032
Diclofenac	0.4±0.033	0.6±0.032	0.7±0.025**	0.8±0.030***	0.6±0.032
ME-EtAC 100	0.4±0.034	0.7±0.032	0.8±0.033*	0.9±0.025**	0.7±0.030
ME-EtAC 200	0.4±0.032	0.6±0.031	0.7±0.029**	0.7±0.033***	0.6±0.030

Data are mean ± SE;*p<0.05 **p<0.01 and ***p<0.001 compared with control group determined by one-way ANOVA.

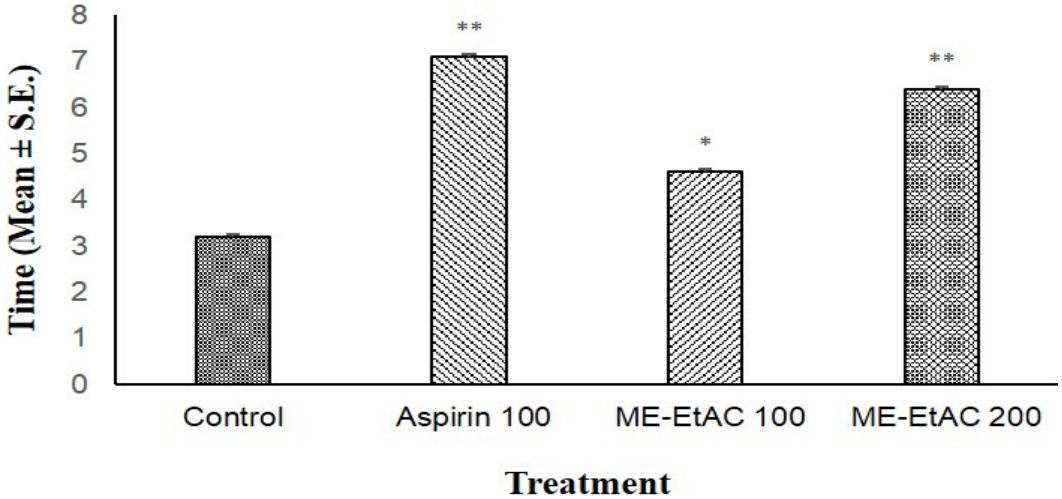


Figure 6: Effects of ME- EtAC on writhing reflex of mice in the writhing test. When mice were intraperitoneally injected with 1% acetic acid (10 ml/kg), the writhing times were counted immediately for 15 min. The experiment was repeated two times. Data are presented as mean ±SD, n=5. *p<0.05, **p<0.01, significance versus control

Table 11: Effects of ME-EtAC on tail immersion assay. When tail was dipped in hot water, the cut off time was 10 seconds. The experiment was repeated two times. Data are presented as mean ±SE, n=5. *p<0.05, **p<0.01, ***p<0.001 significance versus control

Treatment/ Dose mg/Kg	Average tail withdrawing time in second				
	0 min	30 min	60 min	90 min	120 min
Control	1.270±0.04	1.28±0.032	1.263±0. 03	1.273±0.04	1.252±0.04
Aspirin 100	1.202±0.12	2.005±0.05	3.742±0.14***	3.332±0.07***	2.417±0.12**
ME-EtAC 100	1.245±0.07	1.403±0.07	2.905±0.09***	2.427±0.22**	1.855±0.14
ME-EtAC 200	1.223±0.07	1.325±0.09	2.788±0.18***	2.802±0.29***	2.202±0.13**

CONCLUSIONS

The present study introduces the HPLC fingerprinting data of the ethyl acetate extract of leaves of *Myrica esculenta* which will furnish useful information for its identification. Morphological, microscopic and physicochemical measures discussed herein can be regarded as identifying parameters to substantiate and authenticate the drug.

REFERENCES

[1] Jain M, Kapadia R, Jadeja R.N., Thounaojam M.C., Devkar R.V., Mishra S.H. Traditional uses, Phytochemistry and Pharmacology of *Tecomella undulata*: A Review. Asian Pacific Journal of Tropical Biomedicine 2012;1918-23.

[2] Pal KS and Shukla Y. Herbal Medicine: Current Status and the Future. Asian Pacific Journal of Cancer Prevention 4(4):2003; 281-88.

[3] Quality Control Methods for Medicinal Plant Material; WHO (Geneva): 1998, 28-46.

[4] Jeeva S, Lydem FG, Sawian JT and Laloo RC. *Myrica esculenta* Buch.-Ham. Ex D.Don. - a potential ethanomedicinal species in a subtropical forests of Meghalya, northeast India. Asian Pacific Journal of Tropical Biomedicine 2011;174-77.

[5] Chauhan NS. Important medicinal and aromatic plants of Himachal Pradesh 2003, p.192-193.

[6] Chandra K, Chaudhari BG, Dhar BP, Joseph GVR, Mangal AK, Dabur R, et al. Textbook of Database on Medicinal Plants used in Ayurveda, (India): 2007; 207-18.

[7] The Wealth of India: A Dictionary of Indian Raw Material and Industrial Production. CSIR publications, New Delhi, (India):1962; 471-72.

[8] Chatterjee A, Pakrashi SC. The Treatise on Indian Medicinal Plants, Reprinted Edition. Publications and Information Directorate, New Delhi, (India):1941; 32-33.

[9] Kunwar RM & Duwadee NPS. Ethnobotanical notes on flora of Khaptad National Park (KNP) far western Nepal. Himalayan Journal of Sciences 1(1):2003; 25-30.

[10] Panthi MP and Chaudhary RP. Antibacterial activity of some selected folklore medicinal plants from West Nepal. Scientific world 4(4):2006; 16-21.

[11] Mahato RB and Chaudhary RP. Ethanomedicinal study and antibacterial activities of selected plants of palpa district, Nepal. Scientific world 3(3):2005; 26-31.

[12] Kirtikar KR and Basu BD. Indian Medicinal Plants. International book distributors and publishers, Dehradun (India);1918, 268.

[13] Patel KG, Rao NJ, Gajera VG, Bhatt PA, Patel KV and Gandhi TR. Anti-allergic Activity of Stem Bark of *Myrica esculenta* Buch.-Ham. (Myricaceae). Journal of young pharmacist 2(1):2010;74-78.

[14] Jain VK and Jain B. Antihelmentic activity of ethanolic extract of bark of *Myrica esculenta*. International Journal of Pharmaceutical Sciences and Research 1(11):2010; 129-31.

[15] Hong CJ, Mei WY, Mei WD and Song WZ. Preliminary Study on Antioxidative and Radical-scavenging activities of extracts from *Myrica esculenta* Buch.-Ham.Bark, Chemistry and Industry of Forest Products 27:2007; 1-7.

[16] Rawat S, Jugran A, Giri L, Bhatt ID and Rawal SR. Assessment of Antioxidant Properties in Fruits of *Myrica esculenta*: A Popular Wild Edible Species in Indian Himalayan Region. Evidenced based complementary and alternative medicine 2011; Article ID 512787, doi:10.1093/ecam/neq055.

[17] Maddheshiya Y. Textbook of *Myrica esculenta* Bark: Study for Anticonvulsant and Analgesic Activity, Lap Lambert Academic Publishing, U.P. (India);2010, 120.

[18] Agnihotri S, Wakode S and Ali M. Essential oil of *Myrica esculenta* Buch. Ham. compostion, Antimicrobial and Topical Anti- Inflammatory activity. Natural Product Research 26(23): 2012; 2266-69.

[19] Patel T, Dudhpejiya A and Sheath N. Anti inflammatory activity of *Myrica nagi* Linn. Bark. Anc sci life 30 (4): 2011; 100-103.

[20] Patel T, Rajshekhar C and Parmar R. Mast Cell Stabilizing activity of bark of *Myrica nagi.* Journal of Pharmacognosy and Phytotherapy 3(8): 2011;114-117.

[21] Khan Y, Sagrawat H, Upmanyu N and Siddique S. Anxiolytic properties of *Myrica nagi* bark extract. J Pharm Bio 46(10): 2008; 757-61.

[22] Nguyen XN, Phan VK, Chau VM, Bui HT, Nguyen XC, Vu KT, et al. A new monoterpenoid glycoside from *Myrica esculenta* and the inhibition of angiotensin I-converting enzyme. Chem Pharm Bull 58(10):2010; 1408-10.

[23] Joshi S. Practical Pharmacognosy. Frank brothers and co. (publishers) ltd, First edition: 2009,p.72.

[24] Khandelwal KR. Practical Pharmacognosy, 19th edition, Nirali Prakashan, Pune, (India): 2008,p.13-48.

[25] Jain M, Kapadia R, Albert S & Mishra SH. Standardization of *Feronia limonia* L. leaves by HPLC, HPTLC, Physico-chemical and histological parameters, BLACPMA 10(6): 2011; 525-35.

[26] Kumar SK, Sharma G, Sidiq T, Khajuria A, Jain M, Bhagwat D, et al. Immunomodulatory potential of a bioactive fraction from the leaves of *Phyllostachys bambusoides* (Bamboo) in Balb/C Mice. EXCLI Journal 13(6):2014;125-38

[27] Ayoola GA, Coker HAB, Adesegun SA, Adepoju- Bello AA, Obaweya K, Ezennia EC, et al. Phytochemical Screening and Antioxidant activities of some selected Medicinal Plants used for malaria therapy in Southwestern Nigeria, Tropical J Pharm Res 7(3): 2008;1019-24.

[28] Hakim A, Tajuddin, Ghufran A and Nasreen J. Evaluation of anti-inflammatory of the pods of iklil-ul-malik (*Astralagus hamosus* Linn.), IJNPR 1(1): 2010; 34-37.

[29] Koster R, Anderson M and de Beer EJ. Acetic acid for analgesic screening. Fed Proc 18: 1959;412–16.

[30] Bose A, Mondal S, Gupta JK, Ghosh T, Dash GK. Analgesic, anti-inflammatory and antipyretic activities of the ethanolic extract and its fractions of *Cleome rutidosperma*. Fitoterapia 78:2007;515-20.