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Chemical Composition and Antimicrobial Profiling of *Myrica sapida* Wall.: An Important Wild Fruit Species of Uttarakhand Himalaya

Abstract **: The chemical composition and antimicrobial activity of the extracts of the leaves and bark of *Myrica sapida* (MS) were determined. The profiling of the chemical composition of the methanolic extracts of leaves and bark was performed by GC-MS analysis. The antimicrobial activity of both the extracts was detected by using the disk diffusion method against seven microbial cultures/strains. The major compounds identified in the leaves are lupeol (23.74%), squalene (18.02%), methyl linolate (14.85%), pentadecanoic acid (5.41%) and methyl commate D (5.17%), however, in bark extract; linoelaidic acid (50.75%), methyl commate D (10.60%), taraxerone (5.78%), pentadecanoic acid (5.02%), and bis(2-ethylhexyl) phthalate (5.00%). The extracts of both leaves and bark showed significant antimicrobial activity against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Candida tropicalis* and *Candida glabrata*.**

Keywords: ***Myrica sapida,*** **chemical composition, GC-MS analysis, antimicrobial activity.**

Myrica is an important genus of the Myricaceae family with approximately 97 species distributed in both temperate and subtropical regions of the world[1]. The plant list currently includes a total of 121 species in the genus *Myrica*, among which 18 are accepted names, 59 are synonyms and remaining 44 are unassessed[2]. *Myrica sapida* Wall. (MS) is one of the most important members of this genus. It is also known as Kaphal/ Katphala in Uttarakhand and in Indian Himalaya. It is a sub-temperate evergreen dioecious tree with an altitudinal range of 900 – 2,100 m above mean sea level[3]. Apart from Indian Himalaya, it is found in Nepal, China, Pakistan, and Malaya Island. The fruits of this species are recognized as one of the tastiest wild fruits of the sub-Himalayan region and are accepted for its nutritional and therapeutic potential[4, 5].

The edible fruits of MS are sold in the local market. The tree is used as timber, fuel, fodder, wood as well as for tanning and obtaining yellow colored dye. MS serves as a good source of extra income to local communities. Fruits of the plant are also used to prepare jams, syrups, refreshing drinks and pickles[5]. It is rich in vitamin C and polyphenolic compounds such as tannins, phenols, flavonoids and flavonols[6, 7]. Ethnobotanically, MS has been used in the cure of different diseases and disorders including chronic cough, asthma, ulcers, headache, toothache, wounds, joint pain, paralysis, and mental illness[8]. Traditionally, MS is a well recognized medicinal plant and used in different Ayurvedic and Unani formulations such as *Chwayanprash* and *Brahmarasayan*[9] and have contains high mineral content including Na, K, Ca, Mg, Fe, Zn, Mn, Cu, etc[10].

Previously, active compounds like gallic acid, tannin, ascorbic acid, catechin, chlorogenic acid, and ρ-coumaric[11] were isolated from its fruits. Gallic acid, myricanol, myricanone, epigallocatechin 3-O-gallate, stigmasterol, lupeol and oleanolic acid, palmitic acid, β-bisabolene, α-eudesmol acetate and n-hexadecanol[12, 13, 14] were isolated from its bark. Nerolidol, α-pinene, α-selinene, β-caryophyllene, β-selinen, α-caryophyllene, α-cadinol and linalool[15] were isolated from the leaves of the MS. Hence, due to these medicinal properties, it is important to investigate the chemical composition and antimicrobial activities of leaf and bark extracts of MS. Therefore, in the present study we attempted the chemical and antimicrobial profiling of the methanolic extracts of MS collected from Uttarakhand Himalaya.

Materials and Methods : **Plant Collection and Extraction :** Fresh leaves and bark samples of MS were collected from Tehri district of Uttarakhand Himalaya, India from the height of 1,600 m above mean sea level. The plant samples were authenticated by the taxonomist of Botanical Survey of India, Dehradun (Uttarakhand) India and a voucher specimen (BSI/NRC-115223) is preserved in the herbarium of B.S.I., Dehradun. Ten gm of dried powder of leaves and bark was extracted with 100 ml of methanol (80%) using a Soxhlet extractor for 6 h at 70°C. The collected filtrate was dried and concentrated using a rotary vacuum evaporator (Strike-12, Steroglass, Italy) and used for further analyses (preliminary detection of phytochemicals, GC-MS analysis, and antimicrobial analysis).

Gas Chromatography–Mass Spectrometry (GC-MS) Analyses : GC-MS analyses of the methanolic extracts

of MS were performed at University Science Instrumentation Centre, Jawaharlal Nehru University (JNU), New Delhi (India). The analyses of the methanolic extracts were carried out on a GC-MS-QP2010 Plus (Shimadzu, Kyoto, Japan). The system was equipped with an auto injector (AOC-20i), head space sampler (AOC-20s), a mass selective detector with an ion source (220°C) and an interface (260°C). Rtx-5 MS capillary column (Restek Company, Bellefonte, USA) having 30 m (length) × 0.25 mm (diameter) × 0.25 μm (film thickness) was used for GC-MS analyses. The mass range of 40–650 m/z with 1000 ev of threshold was used. The injector was set in the split injection mode having 250°C of temperature. The starting temperature was adjusted to 80°C (3 min), which afterwards increased to 280°C with a ramp rate of 10°C/min. Helium (> 99.99%) with 40.5 cm/s of linear velocity was employed as a carrier gas. The system was programmed with 16.3 ml/min of total flow rate and 1.21 ml/min of column flow according to stranded methods[16, 17, 18]. The compounds of MS extracts were identified by comparing their retention times (RT) and mass spectral fragmentation patterns with the standard database of WILEY8.LIB and NIST11.LIB.

Antimicrobial Profiling : The antimicrobial activity of the leaves and bark extracts of MS was evaluated by the disc diffusion method[9]. Seven pathogenic bacterial and fungal strains were used in this study. These are *Escherichia coli* (MTCC 6908), *Klebsiella pneumonia* (MTCC 6908), *Pseudomonas aeruginosa* (MTCC 2295), *Staphylococcus aureus* (MTCC 3160), *Candida albicans* (MTCC 3017), *Candida tropicalis* (MTCC 3416), and *Candida glabrata* (MTCC 3019). The reference microbial strains were obtained from the microbial type culture collection (MTCC), Indian Institute of Microbial Technology, Chandigarh, India and were maintained at 4°C on slants of nutrient agar (NA) (Merck, Germany). The antimicrobial activity was assessed by measuring the zone of inhibition (ZOI) surrounding the disk and each test was repeated three times and the data were represented as average. Ampicillin (1mg/ml), fluconazole (1mg/ml) and dimethyl sulfoxide (DMSO, 10%) were used as positive and negative controls, respectively.

Results : The yields of the extracts of the leaves and bark were found to be 15.70% and 18.80%, respectively. The GC-MS analysis revealed the presence of tannin, flavonoids, glycosides, steroids, terpenes, saponins, alkaloids and mucilage in methanolic extracts. Carbohydrates were additionally present in the leaf extract. Forty four compounds in the leaf extract and thirty one compounds in the bark extract were identified, representing 97.23% and 97.12% of the total volatile extracts, respectively. In the leaf extract, lupeol (23.74%), squalene (18.02%), methyl linolate (14.85%), pentadecanoic acid (5.41%) and methyl commate D (5.17%) were the major compounds. However, in the bark extract, linoelaidic acid (50.75%), methyl commate D (10.60%), taraxerone (5.78%), pentadecanoic acid (5.02%), bis(2-ethylhexyl) phthalate (5.00%) and linoleic acid (3.02%). Additionally, the following minor compounds (%) were identified in the bark extract: Urs-12-en-28-al (1.9%), sitostenone (1.41%), β-sitosterol (1.36%), methyl commate A (1.3%), α-amyrin (1.07%), 14,17-Nor-3,21-dioxo-.beta.-amyrin, 17,18-didehydro-3-dehydroxy (0.97%), tetradecyl acrylate (0.9%), atraric acid (0.83%), phthalic acid (0.77%), hexadecanal (0.65%), neophytadiene (0.62%), 9,19-Cyclolanost-6-en-3-ol, acetate (0.61%), eicosyl heptafluorobutyrate (0.55%), calarene epoxide (0.55%), pentadecanal (0.45%), methyl linoleate (0.44%), methyl oleate (0.38%), methyl palmitate (0.35%), 9-icosene (0.34%), tetracosanol (0.29%), butyl octyl phthalate (0.27%), octadecanal (0.25%), α-octadecene (0.24%), diisobutyl phthalate (0.23%) and 9-octadecene (0.22%), respectively.

In the leaf extract, the following minor compounds were present: Vitamin e (3.12%), lupenone (2.62%), solanesol (2.25%), phytol (2.16%), β-amyrin (2.07%), pyrogallol (1.81%), β -sitosterol (1.48%), quinic acid (1.42%), stearic acid (1.3%), bis(2-ethylhexyl) phthalate (1.12%), Geranylgeraniol (1.04%), delta-tocopherol (0.94%), 5-hydroxymethylfurfural (0.81%), pyranone (0.79%), tert-butyl 1-[4-(2,6-ditert-butyl-4-methoxyphenoxy)-3-nitro-4-oxobutyl]-2-pyrrolidinecarboxylate (0.72%), α -sringene (0.66%), tetradecyl acrylate (0.65%), neophytadiene (0.63%), melamine (0.52%), methyl palmitate (0.46%), tetraprenol (0.39%), geranyl linalool b (0.39%), acetin (0.33%), 4,8,12,16-tetramethylheptadecan-4-olide (0.29%), lauric acid (0.24%), 1,3-cyclopentanedione (0.19%), vitispirane (0.18%), myristic acid (0.16%), loliolide (0.16%), nerolidol (0.13%), farnesyl acetone (0.12%), methyl linolate (0.11%), stearyl alcohol (0.08%), farnesyl acetate (0.07%), 2-(3-isopropyl-4-methyl-3-penten-1-ynyl)-2-methylc (0.07%), 2,6,10,14-hexadecatetraen, 1-acetoxy-3,7,11,15-tetramethyl-(e,e,e)-(0.06%), hexadecanal (0.2%), geranyl linalool (0.2%) and octadecene (0.1%), respectively. Some of the compounds were not identified and were present in concentrations of < 2%, as auto quantified by the GC-MS analysis. Total of eight compounds were found to be common in both the extracts.

The results of antimicrobial activity of the methanolic extract expressed as the diameter of the zone of inhibition (ZOI) in millimeter are shown in Table 1.

Table 1: Antimicrobial Profile of Methanolic Bark and Leaves Extract of MS

Microbial strains	MSB (Ave ± SD)	MSL (Ave ± SD)	Positive Control (Ave ± SD)	Negative Control (Ave ± SD)
E. coli	10.13 ± 0.47	11.07 ± 0.93	22.32 ± 0.92	0
K. pneumoniae	15.07 ± 0.60	16.17 ± 0.55	24.12 ± 0.51	0
P. aeruginosa	08.50 ± 0.44	09.27 ± 0.64	22.50 ± 0.63	0
S. aureus	12.53 ± 0.95	12.87 ± 1.03	26.17 ±0.78	0
C. albicans	13.20 ± 0.62	13.57 ± 0.75	25.27 ± 0.61	0
C. tropicalis	12.23 ± 0.87	13.03 ± 0.35	22.46 ± 0.89	0
C. glabrata	10.90 ± 0.79	12.73 ± 1.25	21.52 ± 0.47	0

Note: MSB: *Myrica sapida* bark; MSL: *Myrica sapida* leaves.

Discussion : The differences in chemical composition of any plant extract are highly influenced by several factors, such as the genetic and seasonal variation, geographical origin, the plant part used for the study, the time of collection, extraction method, time duration, and solvent system, etc[18, 20]. In the present study, preliminary analysis of phytochemicals reveals the presence of tannin, phenol, flavonoid, glycoside, saponin and other compounds which were found in previous studies[6, 7]. GC-MS analyses of leaf and bark extracts of MS showed the presence of 44 and 31 compounds, respectively. Eight compounds were the common for both the extracts and the major components, linoelaidic acid (RT 16.72; 50.75%), lupeol (RT 29.24; 23.74%), squalene (RT 25.86; 18.02%), methyl linolate (RT 16.73; 14.85%), methyl commate D (RT 36.89; 10.60%), taraxerone (RT 36.09; 5.78%), and pentadecanoic acid (RT 15.03; 5.41%) were found in both the extracts. Lupeol is a triterpenoid which possess anticancer and anti-inflammatory activities[21]. Taraxerone is known for its anticancer, anti-leishmanial and anti-tumour activities[22, 23]. Squalene is a natural lipid known for its anticancer and cosmetic industry use[24]. These components were already reported for their different biological activities.

Pathogenic microbial strains are considered globally as a major threat to health as the commercial drugs in the market are not effective against them[25]. Historically, essential oils and crude extracts of different plant species have been reported to have antiseptics, antimicrobial, antioxidant like different biological properties[26]. It is important now-a-days to investigate scientifically those plants, which have been used in traditional medicines as a potential sources of novel components for the different biological properties[27]. In the present study, ZOI of MS extracts for bacterial and fungal strains were recorded from a range of 08.50 ± 0.44 to 16.17 ± 0.55 mm and 10.90 ± 0.79 to 13.57 ± 0.75 mm, respectively. Previously, the antimicrobial profile of the essential oil of bark extract of MS had been reported[14].

In another study, antimicrobial activity of methanolic bark extract of MS was detected against ten different strains, *E. coli, K. pneumonia, P. aeruginosa*, *Enterococcus species, Salmonella typhi*, *Salmonella paratyphi*, *Proteus mirabilis, S. aureus*, *C. albicans* and *Trichoderma viridae.* The plant extract was effective against all the strains except *S. aureus* and *S. paratyphi*. The ZOI was recorded from a range of 06.83 to 14.50 mm, respectively[28]. The ethanolic fruit pulp extract of MS possess significant antimicrobial activity (ZOI: 6 to 16 mm) against *Escherichia coli* and *Streptococcus pyogenes* except other strains[29]. In the present study, both the extracts of MS showed the much similar activities compared to previous studies.

Conclusion : Detection of preliminary phytochemicals and GC-MS analyses of MS (leaves and bark extracts) confirmed the presence of different biologically active compounds. These compounds have been already reported for their biological activities. Therefore, it can be concluded that the methanolic extracts of MS (leaves and bark) can be used in pharmacological and industrial applications as they possess several active compounds.

Abbreviations : MS: *Myrica sapida*; GC-MS: Gas chromatography- mass spectrometry; ZOI: zone of inhibition; MTCC: Microbial type culture collection; DMSO: Dimethyl sulfoxide; mm: millimeter; RT: retention time.

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