IJPSR (2015), Vol. 6, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 February, 2015; received in revised form, 03 April, 2015; accepted, 08 June, 2015; published 01 September, 2015

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SEED OIL OF *BROUSSONETIA PAPYRIFERA* (L.) VENT

N. Naveen Kumar [1], H. Ramakrishnaiah [*1], V. Krishna [2] and A. P. Deepalakshmi [1]

Department of PG Studies and Research in Biotechnology [1], Government Science College, Bangalore 560001, Karnataka, India
Department of PG Studies and Research in Biotechnology and Bioinformatics [2], Kuvempu University, Shankaraghatta 577451, Karnataka, India

Keywords:

Broussonetia papyrifera, caryophyllene, fatty acid, GC MS, hexadecanoic acid

Correspondence to Author:
H. Ramakrishnaiah

Head
Department of PG Studies and Research in Biotechnology,
Government Science College,
Bangalore 560 001, Karnataka, India

E-mail: hramabt@yahoo.com

ABSTRACT: The current study focuses on evaluating chemical composition and antimicrobial activity of seed oil of *Broussonetia papyrifera*; a widely used Chinese medicinal plant. The chemical constituents of the oil were evaluated by GC-MS analysis. Antimicrobial activity and minimum inhibitory concentrations were determined by disk diffusion and agar dilution methods. The seed oil contains 46 different phytoconstituents where major compounds were hexadecanoic acid (43.6%), heptadecene-8-carbonic acid (17.5%) and caryophyllene (8.4%). Seed oil exhibited inhibitory effect on *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus cereus*, and *Enterobacter aerogenes*, while it showed no such inhibitory effect on fungal strains. The studies revealed that majority of the compounds in oil were saturated fatty acids and their derivatives. The oil possessed significant antibacterial activity against selected bacterial strains while tested fungal strains were completely resistant to *B. papyrifera* seed oil.

INTRODUCTION: Essential oils have been used in the manufacture of perfumes, deodorants, cosmetics and pharmaceuticals. Since various plant based essential oils and their derivatives are extensively used in different industries, proper characterization of essential oils is utmost important. Besides, chemical characterization, bioactivity assays have become an integral part of phytochemical research. Most essential oils have high therapeutic value and therefore employed in treating number of ailments by means of inhalation [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(9).3954-60

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(9).3954-60

Oils extracted from different plants have profound applications even in treating coronary heart diseases [2], hypertension [3], hyperglycemia [4], and cancer [5-7]. Many oils are used for their relaxant, stimulant, depressant, and antiviral properties[8]. Studies have indicated the application of essential oils as antimicrobial agents against a wide spectrum of pathogenic bacteria and fungal strains [9-12].

For India, *Broussonetia papyrifera* (L.) Vent. is an introduced species belonging to the family Moraceae. It is widely distributed in China, Korea, Japan and Thailand. Plant is significantly used in traditional Chinese medicine. It is said to be astringent, diuretic, tonic, vulnerary, diaphoretic and laxative [13, 14]. The fruits are used in the treatment of impotency and ophthalmic disorders[15].

B. papyrifera possesses significant anti-inflammatory, antinociceptive, anti-hepatotoxic, antimicrobial, cytotoxic, and antioxidant activities [16-22]. The leaves and fruits of *B. papyrifera* are reported to have volatile oils[23, 24]. However, no studies are conducted on the chemical properties of seeds of *B. papyrifera*. In view of this, the present study was aimed at evaluating chemical constituents and antimicrobial activity of seed oil.

MATERIALS AND METHODS:

Plant material:

The specimen collected was identified as *Broussonetia papyrifera* (L.) Vent. using Flora of Bangalore [25] and further authenticated by National Ayurveda Dietetics Research Institute, Bangalore (voucher specimen - RRCBI/MCW/09). Separate voucher specimens were maintained in the herbarium of the research center. Fruits of *B. papyrifera* were collected and seeds were separated, processed, and stored for further study.

Oil extraction:

About 100 g of dried seed powder was subjected to hydrodistillation for 8 hours in a Clevenger apparatus. The extracted seed oil was collected by solubilizing in hexane. At room temperature, hexane was allowed to evaporate completely. The hydrodistillation extractions were repeated several times and yield was pooled and stored at 4 °C.

Gas chromatography – mass spectrometry:

GC-MS analysis was performed on Shimadzu GCMS-QP-2010S apparatus using a RTX-5 column (30 m × 0.25 mm; 1.0 µm film thickness). The carrier gas was Helium at a flow rate of 1.0 mL/min. Oven temperature was programmed at 150 °C (1 min), 150 – 280 °C at 5 ºC/min and 280 °C (15 min). The injection port was set at 150 °C. Significant quadrupole MS operating parameters included interface temperature 280 °C, electron impact ionization at 70 eV, ionization temperature of 200 °C and with scan mass range of 40 – 600 *m/z* at a sampling rate of 1.0 scan/s. The compounds were identified by comparing retention time and mass spectra with the MS literature data, NIST, and WILEY library [26-28].

Antimicrobial activity: Microbial cultures: The oil was tested against thirteen bacteria and four fungal strains. Bacteria included *Escherichia coli* NCIM 2931, *Staphylococcus aureus* NCIM 5022, *Salmonella abony* ACM 5080, *Enterobacter cloacae* NCIM 2015, *Klebsiella pneumoniae* NCIM 2957, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* NCIM 2027, *Bacillus cereus* NCIM 2155, *Bacillus subtilis* NCIM 2480, *Bacillus polymyxa* NCIM 2540, *Enterobacter aerogenes* NCIM 2340, *Salmonella spp.* MTCC 1168 and *Salmonella typhi*. Antifungal assay was conducted on *Candida tropicalis* MTCC 184, *Candida albicans* MTCC 183, *Candida glabrata* MTCC 3019 and *Saccharomyces cerevisiae* NCIM 3044.

The microorganisms were procured from American Type Culture Collection (ATCC), National Collection of Industrial Microorganisms (NCIM), Australian Collection of Microorganisms (ACM) and Microbial Type Culture Collection (MTCC) institutes. Bacterial strains were maintained on Nutrient agar whereas fungi on Sabouraud dextrose agar.

Disc diffusion assay:

Antimicrobial activity of the oil was determined by disk diffusion method[29]. The culture media used in the assay were Mueller Hinton agar for bacteria and Sabouraud dextrose agar for fungi. The agar medium was poured into the petriplates to a uniform depth of 4 mm and allowed to solidify. Overnight grown inoculums were adjusted to 0.5 Mc Farland and were spread over the surface of media, using a glass spreader to ensure the confluent growth of the organism. Sterile discs of 6 mm size were then placed aseptically on the surface of the agar media. Aliquots of 15 µL were impregnated on to the discs.

Gentamicin (10 µg/disc) and Amphotericin B (20 µg/disc) were used as positive controls for bacteria and fungi respectively. The inoculated plates were incubated for 24 hours at 37 °C for bacterial strains and for 48 hours at 30 °C for fungi. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was performed in triplicates. The activities were expressed as resistant, if the zone of inhibition was less than 8 mm, intermediate (9-11 mm) and sensitive if more than 12 mm [30, 31].

Minimum Inhibitory Concentrations:

The minimum inhibitory concentration (MIC) values were determined by agar dilution method[29]. Different aliquots of seed oil were added aseptically to molten Mueller Hinton agar media to prepare agar containing oil with concentrations 0.25%, 0.5%, 1%, 2%, and 4% (v/v). The resulting agar solutions were vortexed, poured into sterile petridishes, and allowed to set for 30 minutes. The plates were then inoculated with bacteria that were inhibited in disc diffusion assay. Plates were incubated at 37 °C for 24 hours and then observed for presence or absence of growth. From the results, the MIC was determined as the lowest concentration of oil required to inhibit the growth of the microorganism.

RESULTS AND DISCUSSION:

Chemical composition:

The hydrodistillation of the seeds yielded 1.1% (w/w) pale yellow oil. The chemical compounds and their relative percentage resulted from GC-MS analyses are listed in **Table 1**, in the order of their elution. The seed oil revealed presence of 46 compounds accounting for 98.3% of the oil. About 74.2% of the total seed oil was fatty acids and their derivatives, while 24.1% were volatile essential oil components. The major constituents of seed oil were hexadecanoic acid (43.6%), heptadecene-8-carbonic acid (17.5%) and caryophyllene (8.4%). Caryophyllene was the major sesquiterpene found in the oil besides traces of their derivatives. Compounds such as caryophyllene oxide, alpha-humulene, humulene oxide, alpha-selinene, beta-selinene, 9-octadecenoic acid, and tetradecanoic acid were present in excess of 1%.

Some of the medicinally important compounds were tetradecanoic acid, dodecanoic acid, octadecenoic acid, linoleic acid, and sesquiterpenes such as caryophyllene, humulene, and selinine.

Fatty acids and their derivatives accounted for major part of the seed oil. Hexadecanoic acid, commonly known as Palmitic acid was present at a whopping 43.6%. It is reported that oil extracted from leaves and fruits of *B. papyrifera* also contained hexadecanoic acid and its derivatives in higher quantities [23, 24]. Palmitic acid, a saturated fatty acid in different forms is used as a release agent, natural additive in food products and other organic products. They are also used in the manufacture of detergents and cosmetics. Paliperidone palmitate, synthesized using the oily palmitate ester as a long-acting release carrier medium is used in the treatment of schizophrenia[32-34].

Hexadecanoic acid ethyl ester acts as antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavour, haemolytic, and 5-alpha reductase inhibitor[35]. Octadeca-9,12-dienoic acid (linoelaidic acid**)** is a geometric isomer of linoleic acid and is found in partially hydrogenated vegetable oils. Though present in less than 5%, other fatty acids like tetradecanoic acid, dodecanoic acid, octadecenoic acid, linoleic acid, and their derivatives are known to have medical and industrial applications[36-40].

Earlier studies on oil extracted from leaves of *B. papyrifera* revealed that oil was rich in fatty acids and contained octadecanoic acid, hexadecanoic acid, eicosanoic acid, and 6,10-dimethyl-2-undecanone in higher concentrations[23]. Supercritical carbon dioxide extraction of chemical constituents of oil from fruits and their GC-MS analysis showed presence of fatty acids and their derivatives like 10,13-octadecadienoic acid, Me palmitate, Me stearate and Me oleate as major constituents [24]. The present study revealed that the seed oil is rich in fatty acids and their different forms.

TABLE 1: CHEMICAL COMPOSITION OF SEED OIL OF *BROUSSONETIA PAPYRIFERA*

Compounds	RT (min)	Area %
(-)-beta-Elemene	6.914	0.17
Caryophyllene	**7.638**	**8.41**
alpha-Guaiene	7.732	0.03
alpha-Humulene	8.226	2.35
Longifolene-(v4)	8.461	0.33
1H-Cycloprop e azulene, decahydro-1,1,7-trimethyl-4-methylene, 1aR-(1a.alpha., 4a.beta., 7.alpha., 7a.beta., 7b.alpha.) -	8.533	0.09

2,4-bis (1,1-dimethylethyl)- Phenol	8.672	0.39
beta-Selinene	8.822	1.13
alpha-Selinene	8.939	1.05
1H-Cyclopenta 1,3cyclopropa, octahydro-7methyl-3-methylene-4-(1-methylethyl)-, 3as(3a.alpha., 3b.beta., 4.beta., 7.alpha) 1,2 benzene	9.175	0.10
delta-Cadinene	9.237	0.14
exo-2-Hydroxycineole	9.641	0.37
Sclareolide	9.766	0.16
Pentadecane	10.058	0.41
(-)-5-Oxatricyclo[8.2.0.0(4,6)]dodecane,, 12-trimethyl-9-methylene, [1R-(1R*,4R*,6R*,10S*)]	10.673	0.30
(-)-Caryophyllene oxide	10.750	6.15
1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR- (1a.alpha., 4.alpha., 4a.beta., 7.alpha.,7a.beta.,7b.alpha.)]-	10.996	0.33
Humulene oxide	11.241	1.68
(-)-Spathulenol	11.404	0.20
Agarospirol	11.535	0.19
Tetracyclo[6.3.2.0e2,5.0e1,8]tridecan-9-ol, 4,4 dimethyl-	11.700	0.66
Hedycaryol	12.017	0.89
6,9,12,15-Docosatetraenoic acid, methyl ester	12.275	0.63
Cyclopropanebutanoic acid, 2- 2- 2- (2-pentylcyclopropyl) methyl cyclopropyl methyl cyclopropyl methyl-, methyl ester	12.551	0.11
8-Methylheptadecane	13.140	0.09
Tetradecanoic acid	13.232	1.44
Heptadecane, 3-methyl	13.417	0.15
Octadecane	13.968	0.52
Isopropyl myristate	14.491	0.34
6,10,14-trimethyl-2-Pentadecanone	14.988	0.12
Pentadecanoic acid	15.215	0.34
1,2-Benzenedicarboxylic acid,-bis(2-methylpropyl) ester	15.731	0.58
Heptadecane	15.929	0.12
Eicosanoic acid, methyl ester	16.497	0.81
2-hydroxy- Cyclopentadecanone	16.905	0.32
Hexadecanoic acid	**17.330**	**43.69**
Dibutyl phthalate	17.573	0.24
Heneicosane	17.840	0.61
Heptadecanoic acid	19.080	0.12
1-Octadecanol	19.537	0.10
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	19.806	0.80
9-Octadecenoic acid (Z)-, methyl ester	19.872	0.48
Methyl linolenate	19.957	0.28
Triacontanoic acid, methyl ester	20.257	0.27
Heptadecene-(8)-carbonic acid-(1)	**20.629**	**17.54**
9-Octadecenoic acid (Z)-	20.932	3.15

RT- Retention time

Seed oil comprises of sesquiterpenes (24.1%) and its derivatives. They are widely used as anticancer, immunosuppressive and anti-inflammatory agents [41]. Caryophyllene and caryophyllene oxide are present in relatively moderate levels in *B. papyrifera* and they are used in the manufacture of medicaments and in the treatment of chronic degenerative and non-degenerative diseases [42]. Considering their inexpensiveness and benign properties, fatty acids are widely used as natural additives in food products and organic compounds. Since the food industries are keen to reduce the use of synthetic additives, oil extracted from seeds of *B. papyrifera* can be employed in maintenance or extension of the shelf life of food products.

Antimicrobial activity:

Of the thirteen bacteria studied, seed oil of *B. papyrifera* inhibited growth of four bacteria viz. *S. aureus*, *P. vulgaris*, *B. cereus*, and *E. aerogenes*. All the four bacteria were found to be sensitive as the zone of inhibitions were more than 12 mm (**Table 2**).

TABLE 2: ANTIMICROBIAL ACTIVITY OF SEED OIL OF *BROUSSONETIA PAPYRIFERA*.

Microorganisms	Zone of inhibition (mm)	
Bacteria	**Gentamicin**	**Oil**
Escherichia coli NCIM 2931	14	-
Staphylococcus aureus NCIM 5022	13	14
Salmonella abony ACM 5080	13	-
Enterobacter cloacae NCIM 2015	13	-
Klebsiella pneumoniae NCIM 2957	14	-
Pseudomonas aeruginosa ATCC 27853	14	-
Proteus vulgaris NCIM 2027	15	12
Bacillus cereus NCIM 2155	20	13
Bacillus subtilis NCIM 2480	24	-
Bacillus polymyxa NCIM 2540	18	12
Enterobacter aerogenes NCIM 2340	17	-
Salmonella spp. MTCC 1168	15	-
Salmonella typhi	19	-
Fungi	**Amphotericin B**	**Oil**
Candida tropicalis MTCC 184	13	-
Candida albicans MTCC 183	12	-
Candida glabrata MTCC 3019	14	-
Saccharomyces cerevisiae NCIM 3044	13	-

n=9, - no inhibition

Minimum inhibitory concentrations of oil against four sensitive bacterial strains were determined by agar dilution method. *Staphylococcus aureus* was inhibited at 1% of oil in agar media, whereas *P. vulgaris*, *B. cereus*, and *E. aerogenes* were inhibited by 2% of seed oil (**Table 3**). Higher inhibition was seen in case of *S. aureus*, with a zone of inhibition measuring 14 mm in diameter and MIC value with 1% of oil concentration. The seed oil did not exhibit antifungal activity on all the four fungal strains studied.

TABLE 3: MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF SEED OIL OF *BROUSSONETIA PAPYRIFERA*.

Bacteria	Concentration of oil (%)				
	0.125	**0.5**	**1.0**	**2.0**	**4.0**
Staphylococcus aureus NCIM 5022	+	+	-	-	-
Proteus vulgaris NCIM 2027	+	+	+	-	-
Bacillus cereus NCIM 2155	+	+	+	-	-
Bacillus polymyxa NCIM 2540	+	+	+	-	-

n=9, + growth, - inhibition

Present study is the first report on chemical composition of seed oil of *B. papyrifera* and its antimicrobial activity. In *B. papyrifera*, so far Papyriflavonol A is the potent antimicrobial agent isolated from the root bark[19]. In the present study, four bacterial strains viz. *S. aureus*, *P. vulgaris*, *B. cereus*, and *E. aerogenes* were found to be sensitive to *B. papyrifera* seed oil indicating the presence of antimicrobial compounds. The study evidenced that the four fungal strains were resistant to the seed oil.

CONCLUSION: Major constituents in the oil extracted from seeds of *B. papyrifera* were saturated fatty acids and their derivatives which are highly useful in food processing industry. Many therapeutically important essential oil components were present in the seed oil. Owing to significant antibacterial activity, seed oil might be a good source for developing disinfectants and antibiotics.

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How to cite this article:
Kumar NN, Ramakrishnaiah H, Krishna V and Deepalakshmi AP: GC-MS Analysis and Antimicrobial Activity of Seed Oil of *Broussonetia Papyrifera* (L.) Vent. Int J Pharm Sci Res 2015; 6(9): 3954-60.doi: 10.13040/IJPSR.0975-8232.6(9).3954-60.