

# Phytochemical Studies and Antimicrobial Screening of Non/Less-Polar Fraction of *Psoralea corylifolia* by Using GC-MS

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**Abstract:** *Psoralea corylifolia* is a well-known medicinal plant, traditionally used against several diseased conditions. The present study was conducted to investigate the phytochemical composition and antimicrobial activity of *P. corylifolia* seeds. Non/less-polar fraction of methanolic seed extract was subjected to gas chromatography-mass spectrometry (GC-MS) for phytochemical analysis. A total of fourteen compounds were identified which include aromatic, sesquiterpenes, furocoumarins, sterols, fatty acid and their methyl esters. The predominant compounds were epoxyaryophyllene (3), isopsoralen (6), psoralen (7) and bakuchiol (9). Identification of these compounds was also strongly supported by Kovat's Retention Indices. Furthermore, the *n*-hexane soluble fraction showed significant antimicrobial activity against several bacterial strains. *P. corylifolia* seeds represented a unique chemical composition with considerable antimicrobial activity which not only validates their traditional medicinal uses but also indicates their potential as a source of natural antimicrobial compounds.

**Keywords:** Seeds, Antimicrobial, Non/less-polar fraction, Retention indices.

## INTRODUCTION

One of the famous Chinese traditional medicinal plant, *Psoralea corylifolia* Linn., commonly known as Bavacha and Babechi has wide distribution in tropical and subtropical areas all over the world, especially in Asia and Southern Africa [1-3]. The great herb is widely used for the treatment of many kinds of skin diseases like leprosy, leucoderma, psoriasis [1, 2, 4-11], eczema, vitiligo [1, 6], and effective for heart problems, asthma and urinary discharge [8]. Phytochemical studies with this plant have demonstrated the presence of a number of phytochemicals which include flavonoids, coumarins and monoterpene phenol [12]. The prime focus of the present study is the identification of phytochemicals in non/less-polar fraction of methanolic extract from seeds of *P. corylifolia* and the therapeutic value of this fraction. It may be noted that prior to this report the identification of compounds of the non/less-polar fraction of the methanol extract of seeds has not been reported.

## EXPERIMENTAL

### General

The Shimadzu GC-17 gas chromatograph equipped with flame ionization detector (GC-FID) and with

less-polar capillary column SPB-5 (45m x 0.53mm ID with 0.50 $\mu$ m film thickness of 5% phenyl and 95% methyl silicone), hooked with Shimadzu workstation Class GC-10 was used for GC-FID analysis. Nitrogen was used as carrier and make-up gas with the flow rate 1.4 and 40 mLmin<sup>-1</sup> respectively. The split injector with a splitting ratio of 1:30 was set at 250°C and the FID was set at 270°C. The analysis was performed with an initial temperature 60°C for 1min, and then ramped at a rate of 8°C/min to a final temperature 240°C with holding time 30min. Kovats retention indices were also calculated for identifying phytochemicals.

The gas chromatography electron impact mass spectrometer (GC-EIMS) studies was performed on a Hewlett-Packard 5890 gas chromatograph equipped with HP-5 (25m x 0.22mmID and 0.25 $\mu$ m film thickness, an equivalent of SPB-5) was joint with a Jeol, JMS-HX 110 mass spectrometer operating in EI mode. The temperature of ion source was set at 250°C and the energy of ion source was set at 70 eV, while helium was used as carrier gas at 1.4kg/cm<sup>2</sup> pressure. The fraction was dissolved in chloroform (1:20). The injection volume was 1.0 $\mu$ L.

### Plant Material

The seeds (2kg) of *P. corylifolia* were purchased from an authentic local herbal store and identified by Prof. Dr. S. I. Ali from Department of Botany, University of Karachi.

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## Extraction

The dried and ground seeds (2kg) of *P. corylifolia* were repeatedly (x3) extracted with methanol at room temperature and the extracts were combined. The combined extract was concentrated under vacuum and partitioned between 90% MeOH and *n*-hexane. The *n*-hexane fraction (PC) was dried over sodium sulphate and concentrated under vacuum (127.3g). A small portion of PC (123mg) was treated with MeOH and HCl for methylation. After work-up the methylated fraction (PC-MS; 100mg) was obtained. The PC and methylated fraction (PC-MS) was subjected to GC-FID and GC-EIMS.

## RESULTS AND DISCUSSION

The non/less-polar (*n*-hexane soluble) fraction of methanolic extract of seeds of *P. corylifolia* and its methylated fraction was analyzed on GC-FID followed by GC-EIMS. The phytochemicals were characterized mainly by mass spectral survey (NIST Mass Spectral Search Program, ver. 2.0g, GC-Library NIST-11) with Automated Mass Spectral Deconvolution and Identification System (AMDIS). The identification of constituents was further supported by Kovat's Retention Indices reported in literature and NIST Library.

For calculation of retention indices available pure *n*-alkanes C6, C8, C10, C12 and C22 were used as

primary reference and to cover the range from C8 to C25 by spiking pure *n*-alkanes in kerosene oil and diesel, used as secondary standard. All these were analyzed on GC-FID followed GC-EIMS under the identical conditions as for the fraction and further confirmed by mass spectral survey (NIST Mass Spectral Search Program, ver. 2.0g, GC-Library NIST-11) with Automated Mass Spectral Deconvolution and Identification System (AMDIS).

The non/less-polar fraction of methanolic extract of seeds of *P. corylifolia* was obtained as a dark brown semi solid (127.3g). Fourteen compounds were identified in this fraction which include aromatic, sesquiterpene, furocoumarins, sterols, fatty acid and their methyl esters. For further confirmation and analysis for presence of any carboxylic acid, fraction PC was subjected to methylation. The methylated fraction (PC-MS) was obtained as a dark brown semi solid (100mg) which was also subjected to GC-MS.

## Activity

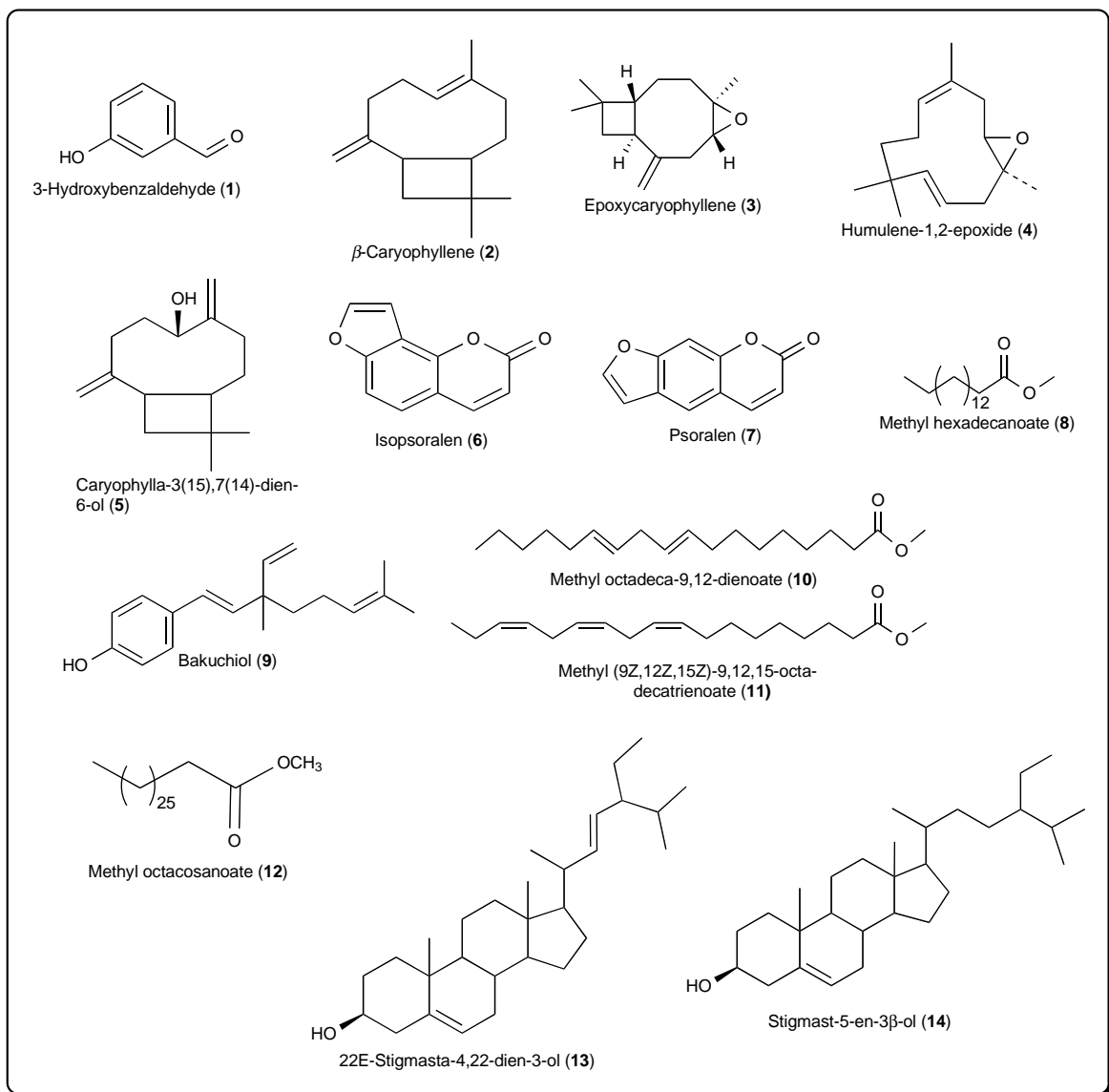
Antimicrobial activity of PC was determined against five different bacterial strains which include, *Micrococcus luetus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* using agar well diffusion method described by National Committee for Clinical Laboratory Standard (NCCLS) [13, 14]. It has been observed that *n*-hexane fraction (PC) was effective

**Table 1: Qualitative and Quantitative Analysis of PC**

S.#	Retention Time (min)	Constituent Name	Chemical Formula	Molecular Weight	Calc. R. I. <sup>a</sup>
1	14.14	3-Hydroxybenzaldehyde (1)	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	122	1359
2	15.5	β-Caryophyllene (2)	C <sub>15</sub> H <sub>24</sub>	204	1405
3	19.47	Epoxyacaryophyllene (3)	C <sub>15</sub> H <sub>24</sub> O	220	1584
4	20.17	Humulene-1,2-epoxide (4)	C <sub>15</sub> H <sub>24</sub> O	220	1607
5	20.97	Caryophylla-3(15),7(14)-dien-6-ol (5)	C <sub>15</sub> H <sub>24</sub> O	220	1632
6	25.45	Isopsoralen (6)	C <sub>11</sub> H <sub>6</sub> O <sub>3</sub>	186	1770
7	26.73	Psoralen (7)	C <sub>11</sub> H <sub>6</sub> O <sub>3</sub>	186	1815
8	28.83	Methyl hexadecanoate (8)	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	1934
9	29.13	Bakuchiol (9)	C <sub>18</sub> H <sub>24</sub> O	256	1955
10	30.97	Methyl octadeca-9,12-dienoate (10)	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	2099
11	31.03	Methyl (9Z,12Z,15Z)-9,12,15-octadecatrienoate (11)	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292	2105
12	44.63	Methyl octacosanoate (12) <sup>b</sup>	C <sub>29</sub> H <sub>58</sub> O <sub>2</sub>	438	-
13	46.99	(22E)-Stigmasta-4,22-dien-3-ol (13) <sup>b</sup>	C <sub>29</sub> H <sub>48</sub> O	412	-
14	49.12	Stigmast-5-en-3β-ol (14) <sup>b</sup>	C <sub>29</sub> H <sub>50</sub> O	414	-

<sup>a</sup>On capillary column with 5% phenyl 95% methyl silicone as stationary phase (DB-5).

<sup>b</sup>Standard not available.



against bacteria cultures tested while no significant change in antimicrobial activity was observed for methylated fraction (PC-MS). However, further investigations are recommended to compare the antimicrobial activity of *P. corylifolia* seeds extracts with commercially available antibiotics.

### Spectral Data of Constituents

#### 3-Hydroxybenzaldehyde (1)

GC-EIMS  $m/z$  (rel. int.);  $C_7H_6O_2$ , 122 ( $M^+$ , 90), 121(100), 93 (42), 73 (5), 69 (5), 65 (45), 43 (7), 40 (6), 18 (19).

#### $\beta$ -Caryophyllene(2)

GC-EIMS  $m/z$  (rel. int.);  $C_{15}H_{24}$ , 204 ( $M^+$ , 21), 175 (10), 161 (34), 148 (29), 134 (24), 120 (42), 109 (21), 105 (47), 93 (100), 79 (50), 67 (33), 55 (39).

#### Epoxycaryophyllene (3)

GC-EIMS  $m/z$  (rel. int.);  $C_{15}H_{24}O$ , 220 ( $M^+$ , 7) 43 (100), 177 (10), 149 (10), 121 (27), 109 (37), 107 (35), 95 (43), 93 (67), 79 (90), 41 (93).

#### Humulene-1,2-epoxide (4)

GC-EIMS  $m/z$  (rel. int.);  $C_{15}H_{24}O$ , 220 ( $M^+$ , 2), 109 (100), 179 (3), 138 (79), 123 (37), 96 (85), 67 (85), 55 (42), 43 (71), 41 (45).

#### Caryophylla-3(15),7(14)-dien-6-ol (5)

GC-EIMS  $m/z$  (rel. int.);  $C_{15}H_{24}O$ , 220 ( $M^+$ , 3), 159 (15), 147 (12), 137 (16), 136 (100), 133 (20), 119 (26), 109 (27), 105 (34) and 93 (44).

#### Isopsoralen (6)

GC-EIMS  $m/z$  (rel. int.);  $C_{11}H_6O_3$ , 186 ( $M^+$ , 12), 185 (100), 158 (52), 130 (10), 102 (14), 79 (4), 51 (10).

**Psoralen (7)**

GC-EIMS *m/z* (rel. int.); C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>, 186 (M<sup>+</sup>, 100), 158 (8), 130 (17), 102 (28), 76 (15), 50 (15).

**Methyl hexadecanoate (8)**

GC-EIMS *m/z* (rel. int.); C<sub>17</sub>H<sub>34</sub>O<sub>2</sub>, 270 (M<sup>+</sup>, 2), 74 (100), 101 (3), 88 (5), 57 (19), 55 (31), 41 (42).

**Bakuchiol (9)**

GC-EIMS *m/z* (rel. int.); C<sub>18</sub>H<sub>24</sub>O, 256 (M<sup>+</sup>, 8), 13 (13), 173 (100), 158 (20), 145 (36), 107 (28), 83 (17).

**Methyl octadeca-9,12-dienoate (10)**

GC-EIMS *m/z* (rel. int.); C<sub>19</sub>H<sub>34</sub>O<sub>2</sub>, 294 (M<sup>+</sup>, 32), 109 (23), 95 (50), 81 (78), 67 (100), 55 (75), 41 (90).

**Methyl (9Z, 12Z, 15Z)-9, 12, 15-octadecatrienoate (11)**

GC-EIMS *m/z* (rel. int.); C<sub>19</sub>H<sub>32</sub>O<sub>2</sub>, 292 (M<sup>+</sup>, 5), 149 (15), 121 (23), 108 (45), 95 (59), 79 (100), 67 (60), 55 (42), 41 (37).

**Methyl octacosanoate (12)**

GC-EIMS *m/z* (rel. int.); C<sub>29</sub>H<sub>58</sub>O<sub>2</sub>, 438 (M<sup>+</sup>, 57), 143 (30), 87 (74), 74 (100), 57 (50); 43 (55).

**22E-Stigmasta-4, 22-dien-3-ol (13)**

GC-EIMS *m/z* (rel. int.); C<sub>29</sub>H<sub>48</sub>O, 412 (M<sup>+</sup>, 18), 55 (100), 394 (20), 271 (26), 255 (66), 159 (40), 133 (40), 95 (45), 83 (90), 69 (65).

**Stigmast-5-en-3β-ol (14)**

GC-EIMS *m/z* (rel. int.); C<sub>29</sub>H<sub>50</sub>O, 414 (M<sup>+</sup>, 30), 329 (10), 213 (13), 145 (20), 107 (30), 55 (37), 43 (100).

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Received on 09-02-2015

Accepted on 02-03-2015

Published on 05-03-2015

<http://dx.doi.org/10.6000/1927-5129.2015.11.23>

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