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**PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF *ATROPA BELLADONNA* AND  
*MATRICARIA CHAMOMILA* OBTAINED BY MICROWAVE EXTRACTION METHOD**

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**ABSTRACT**

Because of the presence of bioactive compounds plant has great medicinal properties. However, proper extraction of the medicinal plants is very crucial in utilization of full potential of medicinal plants. The present study focuses on extraction of two commonly used medicinal plants *Atropa belladonna* and *Matricaria chamomile* by Microwave extraction method. It was observed that the maximum yield of *A. belladonna* was 80 % at power level 800 W in 180 sec while *Matricaria chamomilla* have maximum yield of 60 % when methanol was used as a solvent. GC-MS analysis of the extracts of both the plants indicates that there are many compounds that are well reported for having significant antimicrobial as well as antioxidant activities. Hence these plants are an important source of bioactive compounds.

**Keywords: *Atropa belladonna*, *Matricaria chamomile*, microwave extraction method,  
Phytochemical Analysis**

**INTRODUCTION**

*Atropa belladonna* is perennial plant, that is well known for its medicinal properties Tropane alkaloids which are present in it have spasmolytic and anticholinergic

properties. The other major constituents of *Atropa belladonna* are alkaloids that contain scopolamine and hyoscyamine. Scopolamine is useful for medical purpose and it is of

great value because of its higher physiological activity and has fewer side effects (Evans *et al.*, 1995). Because of the presence of these bioactive compounds this plant has great medicinal properties. These compounds are also anticholinergic agents and hence widely used in Pharmaceutical drugs (Hashimoto *et al.*, 1991; Joshi *et al.*, 2003).

*Matricaria chamomila* is useful to relief various pains, calm the headaches and the tooth aches, to relieve pains of rules and to facilitate the menstruation. It is an anti-inflammatory drug that softens eyelids and eyes (Shivananda *et al.*, 2007; Owlia *et al.*, 2007). Some phenolic compounds like flavonoids, stilbenes, phenolic acid, lignin, lignans and tannins are very common in the flowering tissues, leaves, stem and bark in *M. chamomilla*. (Larson, 1988). Tannins are natural polymeric phenols that contain many phenol rings and have the ability of protein precipitation from solutions. They can inhibit pathogenic bacteria and fungi growth (Molan *et al.*, 1997).

It has been observed that extracts obtained by microwave assisted extraction (MAE) give better results as compared to the traditional Soxhlet extraction method (Sharma *et al.*, 2008). GC-MS is a technique which is commonly employed to analyze the bioactive

compounds preset in plants (Abirami and Rajendran, 2012). The present work was conducted to use the microwave assisted extraction method for the extraction of *A. belladonna* and *M. Chamomilla* and GC-MS analysis of the extracts to identify compounds of significance that can be used in pharmaceutical industry.

## MATERIAL AND METHOD

### Sample Collection

Plant samples *Atropa belladonna* and *Matricaria chamomilla* were collected arbitrarily from northern hilly areas of Pakistan. After collection plant material were weighed before drying.

### Drying and grinding of plants

The roots were collected from selected plants and dried in shady places in Plant Biotechnology Laboratory, Department of Biotechnology, Lahore College for Women University. Then powder of roots was kept for drying over night at 37°C to remove complete moisture. To make powder of roots it was grinded by using grinder. After getting powder form of plants samples the powder is sieved to remove all the particles from it and obtained pure samples. Then the total powder form of roots was again weighed.

### Microwave assisted extraction

Plant materials were extracted by using microwave assisted extraction method. First

of all, dried plant samples were taken in beaker and solvent were added in it.

Various solvents such as ethanol and methanol were used as well as other parameters such as time and power levels were optimized to get the better yield. 5 ml of organic solvent were used during extraction. Initially 0.15 g sample of plant was weighed and put it in 100 ml beaker. Solvent (5 ml) was added to it and beaker was covered by using polythene bag having holes on it to remove inside pressure. In another beaker little amount of silica gel was taken in it. Beaker having sample was placed in beaker containing silica gel and both of the beakers were put inside microwave oven. Time and power level was adjusted. After complete process solvent extract was filtered with filter paper. The obtained filtrate was then poured into china dishes which were pre-weighed and left for drying at room temperature. After evaporation of solvent completely, then scratched dried extract by using common pin or blade and again weighed. Then 2 ml of solvent dissolved in it again and stored these extracts in vials at 4°C in refrigerator. In the same way all plant samples were extracted.

#### **Percentage Yield**

After extraction the percentage yield of plant extract was found out by using the formula

$$\text{Weight of sample} = (\text{weight of china dish} + \text{extracts}) - (\text{Weight of china dish})$$

Content of plant in the extracted sample determined by the weight difference:

$$\text{Plant sample content (\%age)} = \frac{\text{amount of sample extracted (g)}}{\text{weight of original sample (g)}} \times 100$$

#### **Chemical analysis**

GC-MS analysis of the methanolic extract of *Atropa belladonna* and *Matricaria chamomilla* was performed by using a GC-MS system SHIMADZU Model no. GCMS 2010 Japan. An electron ionization system was operated with 70eV ionization energy for GC-MS analysis. At constant flow rate, Helium gas which was used as carrier gas. The injecting temperature was 250°C and the temperature of ion source was maintained at 200°C, the temperature of oven was set at 110°C, with the increase of 10°C per minute to 200°C, then 5°C per minute to 280°C, which ends up with 9 min isothermal temperature at 280°C. Mass spectra were obtained in a scan interval of 0.5 seconds, at 70 eV and fragments which was from 45-450Da. The delaying of solvent was 0-2 min and 36 min was the total time of GC-MS running. Data was collected by using software Lab-Solution (Ezhilan and Neelamegam, 2011).

#### **RESULTS AND DISCUSSION**

The present study deals with the microwave assisted extraction of *Matricaria chamomilla* and *Atropa belladonna* changing different parameter like power and temperature. Then these extracts were used for GC-MS analysis.

#### **Microwave assisted extraction of plants**

In present study, plant samples of both the plants *Atropa belladonna* and *Matricaria chamomilla* were extracted with methanol and ethanol solvents by applying different power levels and time. Results have revealed that methanol has maximum yield of *A.belladonna* 80 % at power level 800 W in 180 sec while the 73.33 % yield was obtained when ethanol was used as solvent under these conditions. (Table 1) *Matricaria chamomilla* have maximum yield 60 % in methanol solvent and 53.33 % in ethanol solvent at power level 800 W and time 180 sec (Table 2). Hence, the power level of 800 W is effective for extracting maximum yield and time of 180 s was found to be optimum for both the plants.

In present study, plant samples were extracted with methanol and ethanol solvents by changing time and different power levels. It has been reported that alcoholic extracts are a better solvent for extraction of substances that are antimicrobial active as compared to water and hexane (Guerin-Fauble, 1996)

Results indicated that methanol has better yield than ethanol. Also the power level of 800W is effective for extracting maximum yield and time of 180s was overall suitable. It has been investigated that MAE requires less time, greater extraction rate, less solvents, and improved products with minor loss (Fulzele and Satdive, 2005; Badami et al., 2007). In another study ( Mattima et al., 1997; Joshi et al., 2009) reported that in MAE, reduced time not only has the economic advantage but there is also fewer risk of oxidation and decomposition of phytochemical. Microwave assisted extraction is hence a good option for the heat sensitive materials.

#### **GC-MS analysis**

During present study the bioactive compounds which are identified by GC-MS study from methanolic extract of *Atropa belladonna* are fatty acids, steroidal saponins, and alcoholic compounds like n-pentadecanoic acid, n-hexadecanoic- methyl ester, octadecanoic acid, oleic acid, dodecanol, Neodiosgenin, 1-hexdecanol, 1-heptadecanol. (Table 3) These compounds could be reason of antioxidant activity of plant. It was observed that methanolic extract of *Matricaria chamomilla* has terpenes, steroids, sesquiterpenes and fatty acids like trans-Z-alpha-Bisabolene epoxide, alpha –

bisabolol, Cycloheptane, Stigmasterol, diosgenin, bis phalate, beta sitosterol, eicosanoic acid.(Table 4).

During the present work the plant extracts were analyzed by GC-MS to identify the compounds present in them. The *Matricaria chamomilla* has antibacterial effects which have been well documented (Mann and Staba, 1986). The constituents of *Matricaria chamomilla* such as luteolin,  $\alpha$ -Bisabolol, apeginin and quercitin, have been reported to have antimicrobial properties. (Ahmed *et al.*, 2010) Terpenes, have shown rising promise *in vivo*, antibacterial activity of number of different species of bacteria (Zwenger and Basu, 2008). *Matricaria chamomilla* have different phenolics compounds. Some phenolic compounds have antioxidant as well as antimicrobial effects (Al-Ismail and Aburjai, 2004). The antimicrobial and antioxidant activity of *M. chamomilla* is mostly because of the phenolic components, mainly the flavonoids, patuletin, quercetin, apigenin, luteolin, and glucosides, but also due to the major compounds of the *M. chamomilla* alpha-bisabolol and its oxides (Hadaruga *et al.*, 2009).

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Table 1: Percentage yield of *A. belladonna* by Microwave assisted extraction method using different solvents

Plant Name	Solvent used	Power level (W)	Time (sec)	Intial wt. (g)	Dry wt. (g)	% yield
<i>Atropa belladonna</i>	Methanol	800	180	0.15	0.12	80
			120	0.15	0.11	73.33
			60	0.15	0.09	60
		600	180	0.15	0.11	73.3
			120	0.15	0.1	66
			60	0.15	0.07	46.66
	Ethanol	800	180	0.15	0.11	73.33
			120	0.15	0.09	60
			60	0.15	0.07	46.66
		600	180	0.15	0.1	66
			120	0.15	0.09	60
			60	0.15	0.07	46.66

Table 2: Percentage yield of *M. chamomilla* by Microwave assisted extraction method using different solvents

Plant Name	Solvent used	Power level (W)	Time (sec)	Intial wt. (g)	Dry wt. (g)	% yield
<i>Matricaria chamomilla</i>	Methanol	800	180	0.15	0.09	60
			120	0.15	0.08	53.33
			60	0.15	0.06	40
		600	180	0.15	0.09	60
			120	0.15	0.07	46.66
			60	0.15	0.05	33.33
	Ethanol	800	180	0.15	0.08	53.33
			120	0.15	0.07	46.66
			60	0.15	0.05	33.33
		600	180	0.15	0.08	53.33
			120	0.15	0.06	40
			60	0.15	0.04	26.66

Table 3 Bioactive compounds indentified in the methanolic extract of *Atropa belladonna* by GC-MS

Sr. No	Compounds	Rt. Time	Nature of compounds
1	n-pentadecanoic acid	12.658	Fatty acid
2	Hexadecanoic acid-methyl ester	13.433	Fatty acid
3	Octadecanoic acid	13.125	Fatty acid
4	Oleic acid	19.867	Fatty acid
5	Dodecanol	20.708	Alcoholic compound
6	Neodiosgenin	28.592	Steroidal Saponins
7	Spirost-5-en-3-yl acetate	33.117	Steroidal Saponins
8	1-hexdecanol	14.767	Alcoholic compound
9	1-heptadecanol	17.683	Alcoholic compound

Table 4: Bioactive compounds indentified in the methanolic extract of *Matricaria Chamomilla* by GC-MS

Sr. No	Compounds	Rt. Time	Nature of compounds
1	trans-Z-alpha-Bisabolene epoxide	10.008	Oxygenated sesquiterpenes
2	Alpha – bisabolol	9.917	Terpenes
3	Cycloheptane	11.400	Cycloalkane
4	Stigmasterol	33.917	Plant sterol
5	Diosgenin	33.158	Steroid
6	Bis-phathalate	12.267	Diester of phathalic acid,
7	Beta sitosterol	33.917	Plant sterol
8	Eicosanoic acid	15.350	Saturated fatty acid