

**COMPARATIVE STUDY OF MAE, SONICATION & CONVENTIONAL METHODS IN
EXTRACTION OF M. SPICATA (DALZ.) NICOLSON****D.G.KARPE* AND S.P.LAWANDE***P.G. Department of Chemistry, Shri Chhatrapati Shivaji College, Shrigonda, Dist-Ahemdnagar-413701 (MS) India.***ABSTRACT**

A conventional method of extraction requires high energy and more time. Soxhlet extraction is thermal extraction method. Microwave assisted extraction (MAE) and sonication methods are new techniques which requires less time and are more efficient than thermal extraction & maceration methods. Microwave is an electromagnetic radiation consists of electric and magnetic field having a frequency ranged from 0.3 to 300 GHZ, it penetrates into certain materials and interacts with polar components to generate heat, and hence selective targeted materials can be heated and extracted. Sonication is sound energy which agitates particles. Ultrasonic energy of 720 KHZ is used for extraction. A MAE and Sonication method gives better yield of extracts within smaller time. In MAE extractive value is better compared with other extraction methods. *Moullava spicata* aerial parts (powdered plant material) extracted in different solvents (Petroleum ether, Ethyl acetate, Methanol) and using different extraction techniques. Extractive value is determined and antibacterial & antifungal analysis was done. Crude extracts obtained from MAE shows better results. Another important application of MAE is that, it was carried out at low temperature hence the chance of decomposition of organic compounds is minimum as compared to thermal extraction.

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INTRODUCTION

Extraction is the process in which organic phytoconstituents (secondary metabolites) are withdrawn from plant material. These secondary metabolites are produced by living organisms. Soxhlet, maceration, percolation are conventional methods of extraction of crude drugs from plants. In present study new methods used for extraction are microwave assisted extraction (MAE) and sonochemical extraction. Microwave and sonication are very safe for extraction¹. These methods are widely used for isolation of different constituents from plants². The advantage of both these methods is the requirement of very short time³. Extraction is the first step in medicinal plant research because crude extracts are used for isolation and purification of chemical components present in the plants. The correct choice of method is most important in the extraction process and better yield of the products within a very short time period is the main advantage of microwave⁴. In microwave assisted extraction power of microwave & solvents used for extraction are variable. In conventional methods of extraction chemical constituents from plant are diffused through the cell walls and then dissolved in solvent. In MAE and sonication they increase the internal kinetic energy of the molecules and sometimes it break the cell walls¹. Faster heating takes place in microwave extraction. During absorption process the microwave energy is converted into kinetic energy. When the liquid absorbs microwave the kinetic energy of its molecules increases, the diffusion rate increases therefore rate of mass transfer increases. In sonication, energy is given in the form of sound; it desorbs the secondary metabolites from the cells and get dissolves in the solvent. Vibration energy can dissociate the molecules into the solvent so it is better technique for extraction. In sonication sound waves are used for agitation of particles. The sound waves used in sonication are usually ultrasound with frequencies of about 20 kHz

(20,000 cycles /sec) and as frequency increases the strength of agitation increases. These vibrations can disrupt molecular interactions and mix molecules into solvents. In both the techniques solvents play an important role. In MAE solvent polarity matters in extraction process.

MATERIALS AND METHODS

All solvents used for extraction are purchased from Molychem pvt. ltd, Mumbai. All solvents are double distilled on a water bath at lower temperatures.

Method of Extraction

(i) Soxhlet Extraction

Dry powdered plant material of *moullava spicata* is extracted in soxhlet apparatus using Petroleum ether, Ethyl acetate, Methanol and 50% Alcohol. Extraction was carried out in soxhlet; thimble used for extraction is made up of whatman filter paper. Extraction temperature and extraction time is variable for every solvent. Solvents are evaporated on rotary evaporator (Buchi, Rotavapor R-3 model). Extractive values are calculated by weighing the crude extracts.

(ii) Microwave Assisted Extraction (MAE)

Fresh powdered plant material of *m.spicata* was taken in small sized soxhlet apparatus and placed in a microwave oven (RAGA's scientific microwave system) . Extraction is carried out in petroleum ether, ethyl acetate, methanol and 50% alcohol.

(iii) Sonication method

Dry powdered plant material of *m.spicata* was taken in round bottom flask; solvent is added in to it and kept in sonicator. Solvents used for extraction are petroleum ether, ethyl acetate, methanol and 50% alcohol. Solvents are evaporated & extractive values are calculated.

Soxhlet Extraction

Solvent used for Extraction	Temperature of	Extraction (in ° C)	Extraction Time
Petroleum Ether	60		06 hours
Ethyl acetate	76		05 hours
Methanol	65		05 hours
50 % Alcohol	100		07 hours

Microwave Assisted Extraction (MAE)

Solvent used for Extraction	Power of M.W. used (in %)	Temp. attained (in ° C)	Extraction Time (in Minutes)
Petroleum Ether	100 (700 W)	62	15
Ethyl acetate	100 (700 W)	61	15
Methanol	100 (700 W)	52	15
50 % Alcohol	100 (700 W)	63	15

Sonication method

Solvent used for Extraction	Extraction time (in Minutes)
Petroleum Ether	15
Ethyl acetate	15
Methanol	15
50 % Alcohol	15

Extractive values are calculated by weighing the extracts. Extracts from Microwave Assisted Extraction (MAE) are screened for antibacterial and antifungal activities.

RESULTS AND DISCUSSION

Extraction of *m.spicata* powdered plant material in different solvents by using various extraction techniques shows following results.

Table I

Method used for Extraction	Extractive values (%)			
	Petroleum Ether	Ethyl Acetate	Methanol	50 % Alcohol
Soxhlet	1	0.60	12.8	0.65
Microwave	0.2	0.5	6.55	4.65
Sonication	0.05	0.15	1.75	1.85

Table II

Antibacterial and Antifungal study of crude extracts by MAE method

ANTIBACTERIAL ACTIVITY TABLE				
MINIMAL INHIBITION CONCENTRATION [MICROGRAM /ML]				
Extract Code	<i>E.Coli</i> MTCC 442	<i>P.Aeruginosa</i> MTCC 441	<i>S.Aureus</i> MTCC 96	<i>S.Pyogenus</i> MTCC 443
MSPMW ^a	12.5	100	125	250
MSEAMW ^b	200	250	125	200
MSMEMW ^c	125	250	200	12.5

a :*M.spicata* Petroleum ether extract by Microwave Extraction

b :*M.spicata* Ethyl acetate extract by Microwave Extraction

c :*M.spicata* Methanol extract by Microwave Extraction

Table III

Minimal Inhibition Concentration [Microgram / MI] for Standard Drugs

Drugs	<i>E.Coli</i>	<i>P.Aeruginosa</i>	<i>S.Aureus</i>	<i>S.Pyogenus</i>
	MTCC 442	MTCC 441	MTCC 96	MTCC 443
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	--	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table IV

ANTIFUNGAL ACTIVITY TABLE			
MINIMAL INHIBITION CONCENTRATION [MICROGRAM /ML]			
Extract Code	<i>C.Albicans</i> MTCC 227	<i>A.Niger</i> MTCC 282	<i>A.Clavatus</i> MTCC 1323
MSPMW	500	>1000	>1000
MSEAMW	>1000	>1000	>1000
MSMEMW	500	>1000	>1000

Minimal Inhibition Concentration [Microgram / MI] for Standard Drugs

Drug	C.Albicans	A.Niger	A.Clavatus
	MTCC 227	MTCC 282	MTCC 1323
Nystatin	100	100	100
Greseofulvin	500	100	100

The time of extraction by Soxhlet extraction is more than six hours for every solvent. By Microwave Assisted Extraction (MAE) time of extraction is very short and the extractive values for polar solvents (Methanol) are good. Sonication extraction gives small extractive values but the chance of degradation of phytochemicals is less because extraction was done at room temperature. The extractive value by sonication method is good for methanol in short minutes. It indicates that MAE extraction is better over all other extraction techniques. Petroleum ether, ethyl acetate and methanol extracts by MAE method screened for antibacterial and antifungal activity. Ampicillin, Chloramphenicol, Ciprofloxacin (Standard antibacterial drugs) having Minimal Inhibition Concentration (MIC) 100 µg/ml, 50 µg/ml, 25 µg/ml respectively for E.Coli (MTCC442), Petroleum ether extract of *m.spicata* by MAE having MIC 12.5 µg/ml for E.Coli, it means petroleum ether extract shows antibacterial activity. Ampicillin having MIC 250 µg/ml for S. Aureus (MTCC96), *m.spicata* petroleum ether, ethyl acetate and methanol extract having MIC 125 µg/ml, 125 µg/ml and 200 µg/ml, it means that all extracts are active against S.Aureus. Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin having MIC 100 µg/ml, 50 µg/ml, 50 µg/ml, 10 µg/ml respectively for S.Pyogenus, *m.spicata* methanol extract having MIC 12.5 µg/ml, it means that methanol extract is active against S.pyogenus. For antifungal study

Nystatin and Gresofulvin are used as standard antifungal drugs. Gresofulvin having MIC 500 µg/ml for C. Albicans, petroleum ether and methanol extracts of *m.spicata* having MIC 500 µg/ml for both, it means they are active against C.Albicans. The present results shows that crude extracts of *m.spicata* are active against various bacterial and fungal species.

CONCLUSION

The present investigation means that MAE is better over all other extraction methods when proper microwave power and polar solvent is used for extraction. Ethyl acetate extract of *m.spicata* by the conventional method of extraction and by microwave assisted extraction shows good antibacterial activity. Methanol extract by microwave assisted extraction shows good antibacterial and antifungal activity. Extracts of *m.spicata* may contain active phytoconstituents.

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