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Comparative study of various methods for extraction of antioxidant and antibacterial compounds from plant seeds

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Abstract

Extracts from seeds of five different plants were prepared in water, methanol, and ethanol by employing five different methods of extraction viz. Soxhlet method, ultrasonication, extraction by continuous shaking at room temperature, and microwave assisted extraction- with and without intermittent cooling. All these extracts were compared with respect to extraction efficiency, total phenol content, total flavonoid content, antioxidant capacity, and antibacterial activity. Soxhlet method proved best in terms of high extraction efficiency, and extraction of phenolic compounds. Microwave assisted extraction with intermittent cooling (MAE), room temperature extraction by shaking (ERT), and ulrasonication assisted extraction (UAE) proved good at extracting antibacterial compounds from plant seeds. Latter also proved effective at extracting antioxidant compounds. Extraction efficiency was found to have no notable correlation with any of the parameters assayed. Methanol proved most suitable solvent for extraction of flavonoids.

Keywords: Extraction, Microwave assisted extraction (MAE), Ultrasonication assisted extraction (UAE), Seeds, Flavonoids

1. Introduction

Extraction as the term pharmaceutically used, can be defined as the technique used for separation of therapeutically desired active constituent(s) and elimination of unwanted insoluble material by treatment with selective solvents [1]. Screening the crude plant extracts for the desired bioactivity is among the most important operations in medicinal plant research [2], and extraction is the first crucial step of the process. To have a complete idea of bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology, so as to achieve maximum possible extraction efficiency [3]. Obtaining better quality and high efficiency of extraction from herbs being significant, one has to optimize the extraction methods for better extraction efficiency. Efficacy of plant extracts may in some cases be dependent on extraction efficiency. A strong positive linear correlation

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between extraction efficiency and total antibacterial activity was found during investigation on antibacterial activity of plant seed extracts [4]. The need for selection of most appropriate extraction methodology is evident from the fact that when different methods are applied on same plant material with same solvent, extraction efficiency can vary significantly. In addition, the method selected as the most appropriate one also needs to be standardized so as to achieve acceptable degree of reproducibility.

The development of modern sample preparation techniques (such as microwave assisted extraction, ultrasonication assisted extraction, supercritical fluid extraction, etc.) has brought significant advantages over conventional methods in terms of reduction in organic solvent consumption and in minimizing sample degradation. Standardization of extraction procedures contributes significantly to the final quality of the herbal drug. Selective separation of the target components from the sample at maximum amount and/or interferences elimination are the main objectives of the extraction processes [5]. To have a complete idea of the bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology to achieve the broadest possible range of phytochemicals. The purposes of standardizing extraction procedures for production of crude drugs are to obtain the therapeutically desired portion and to eliminate the inert material by treatment with selective solvents and methods. With the increasing demand for herbal medicinal products, and natural products for health care all over the world, herbal manufacturers aim at using the most appropriate extraction technologies to produce extracts of defined quality with least batch to batch variation, which can also help in scale-up of extraction. Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material : solvent ratio, etc.

Present study aimed at comparing different extraction method with respect to their ability to extract antioxidant and antibacterial components from plant seeds. Natural antioxidants occur in all parts of the higher plants including seeds [5]. We prepared extracts of five different plant seeds in methanol, ethanol or water by five different methods, and compared the preparations with respect to extraction efficiency, total phenol content, total flavonoid content, antioxidant capacity, and antibacterial activity. Seeds selected were of *Annona squamosa* (Annonaceae), *Manilkara zapota* (Sapotaceae), *Phoenix sylvestris* (Palmae), *Syzygium cumini* (Myrtaceae), and *Tamarindus indica* (Cesalpiniaceae). Common names for these plants are custard apple, cheeku, date palm (khajur), jamun, and tamarind (imli), respectively. Methods employed for extraction were Soxhlet, extraction at room temperature (ERT), ultrasonication assisted extraction (UAE), and microwave assisted extraction with and without intermittent cooling, abbreviated respectively as MAE and MAEC.

2.Materials and Methods

2.1. Plant materials

Seeds of five plant materials *Tamarindus indica* L., *Annona squamosa* L., *Syzygium cumini* L., *Phoenix sylvestris* Roxb., and *Manilkara zapota* L., were procured from the fruits purchased from local market in the city of Ahmedabad and stored in air tight containers. They were authenticated for their unambiguous identity by Prof. Y. T. Jasrai, Head of Botany Dept., Gujarat University, Ahmedabad.

2.2. Test organisms

Pseudomonas oleovorans (MTCC 617) and *Staphylococcus epidermidis* (MTCC 435) were

procured from Microbial Type Culture Collection, Chandigarh.

2.3.Extraction

Dried seeds were powdered to a uniform particle size. Sample to solvent ratio was kept constant for all the methods- 1 g seed powder in 50 ml solvent. Absolute methanol (Merck, Mumbai) and 50 % ethanol (Ureca consumers) were used for extraction.

2.3.1. Soxhlet extraction

It was performed using Soxhlet apparatus for 3 h for all the solvents. Total volume of the system was kept 100 ml.

2.3.2. Extraction at room temperature (ERT)

It was carried out in 250 ml (Borosil) flask on a shaker for 24 h at room temperature. Flask was covered with aluminum foil to prevent any evaporation.

2.3.3. Microwave assisted extraction (MAE) [3]

It was carried out in a microwave oven (Electrolux EM30EC90SS) at 720 W with intermittent cooling (each cooling cycle was of 40 s) in 250 ml screw capped glass bottle (Borosil). Dark (brown) bottles were used to limit effect of light on plant material. Cap of the bottle was kept little loose during extraction. Total duration of microwave heating for extraction in methanol and water was 90 s, whereas for ethanol it was 70 s.

2.3.4.Microwave assisted extraction (continuous) (MAEC)

It was carried out in the same microwave oven at 720 W, without any intermittent cooling in 250 ml screw capped bottle. To minimize evaporation cap was kept little more tight than during MAE. Total duration of microwave heating was 75 s for all solvents.

2.3.5. Ultrasonication assisted extraction (UAE)

It was carried out in an ultrasonicator (6 mm probe; Syclon JY92-11DN) at 720 W in a 100 ml glass beaker. Working frequency was 20-25 KHz with the total treatment time (including several 5 s cycles of working on and working off time) being 2 min for all the solvents. UAE was carried out at room temperature.

Extracts were clarified by centrifugation (Remi BZCI-8729) at 10,000 rpm for 15 min, followed by filtration with Whatman # 1 filter paper (Whatman International Ltd., England). After evaporation dried extracts were reconstituted in respective solvent. For flavonoid assay extracts were reconstituted in 99% methanol, for phenol and antioxidant assay reconstitution was made in 95% methanol. For antibacterial assay extracts were reconstituted in dimethylsulfoxide (DMSO; Merck). Extraction efficiency was calculated as percentage weight of the starting dried plant material. Reconstituted extracts were stored in autoclaved glass vials (15 ml, Merck) under refrigeration (4-8°C). Internal surface of the vial cap was covered with aluminum foil to prevent the leaching of any compounds from the cap into the extract [6].

2.4. Total antioxidant capacity

The molybdate assay used for this purpose is based on the reduction of Mo (VI) to Mo (V) by the sample and subsequent formation of a green phosphate/Mo (V) complex at acid pH [7]. The tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95˚C for 90 min. After the mixture had cooled to room temperature, the absorbance of each tube was measured at 695 nm. The standard curve was prepared by using known concentrations (0.2-14 mM) of gallic acid (SRL, Mumbai). The antioxidant capacity of extracts was expressed in terms of gallic acid equivalent (GAE)/g of dry extract. Ascorbic

acid (2 mM) was used as positive control, which registered a value of 30.80 mM GAE.

2.5. Estimation of total phenolic content

Folin-Ciocalteu method [8] was used to determine total phenolic content of the sample. 0.2 mL of Folin-Ciocalteu reagent (10 % v/v) was added to 0.1 mL of the sample, and was vortexed for 5 min, followed by addition of 0.8 mL of sodium carbonate. This reaction mixture was incubated for 2 h at room temperature. The absorbance was measured at 765 nm. The calibration curve was prepared by employing gallic acid at concentrations of 0.4 to 1.6 mM.

2.6. Estimation of total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination [9]. 0.5 mL of each plant extract was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The reaction mixture was allowed to stand at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by using quercetin (Sd fine chemicals, Mumbai) at concentrations of 12.5 to 100 µg/mL in methanol.

2.7. *Antibacterial activity*

It was carried out using microbroth dilution method [10]. Assay was performed in sterile 96-well microtitre plate (HiMedia). Total volume of the assay system in each well was kept 200 µL. Muller-Hinton broth (HiMedia, Mumbai) was used as growth medium. Inoculum density of the test organism was adjusted to that of 0.5 McFarland standard. Broth was dispensed into wells of microtitre plate followed by addition of test extract and inoculum. All extracts were tested at a concentration of 100 µg/ml. A DMSO control was included in all assays [11]. Gentamicin (HiMedia) served as positive control. Plates were incubated at 35ºC for 16-20 h, before being read at 655 nm in a plate reader (BIORAD 680). Inhibition of growth was expressed in percentage of growth in DMSO control.

2.8. Statistical analysis

ANOVA was done for all data sets with MS®-Excel. In ANOVA the null hypothesis (that all methods are equivalent) will not be rejected only if there is no significant difference between any pairs of the means. On other hand, it would be rejected even if there is a significant difference between one pair of means. Therefore it becomes necessary to identify which of the pairs differ significantly and which do not. For this the method given by Snedecor and Cochron in 1959 explained as *Q test* was applied [12]. All the experiments were set in triplicate, and results were recorded as their mean ± SD.

Table 1. Results of different assays for *A. squamosa* seed extracts

Solvent	Method	Extraction efficiency (%)	Total flavonoid (mg/ml QE/g of dry extract	Total phenol (mM GAE/g of dry extract)	Antioxidant capacity (mM GAE/g of dry extract)	Antibacterial activity (% inhibition at 100 µg/ml) dry extract)	
						S. epidermidis	***P. oleovorans***
Water	Soxhlet	12.25±0.91	Not detectable	102.08±8.83	804.15±253.37	0.00±0.00	19.82±1.27
	ERT	6.26±0.49	2.73±0.22	2317.51±25.80	3666.65±261.89	48.21±2.52	33.57±11.10
	MAE	9.13±0.76	3.87±1.56	368.85±32.60	1123.06±152.29	75.23±3.53	29.29±3.02
	MAEC	10.63±0.05	2.55±0.25	1225.67±192.59	305.18±82.65	12.76±1.68	18.02±1.27
	UAE	12.33±0.92	13.10±0.97	1113.39±58.32	1134.00±87.47	55.08±4.10	22.31±7.61
Methanol	Soxhlet	12.23±0.50	02.83±0.00	2529.52±129.30	1790.41±377.10	48.49±0.00	0.00±0.00
	ERT	11.66±0.05	16.62±02.94	999.01±156.98	787.03±327.36	43.18±16.07	30.72±3.02
	MAE	7.56±0.40	45.84±05.53	2390.81±57.31	939.07±107.68	44.70±20.35	37.86±3.02
	MAEC	8.16±0.70	43.04±03.76	2206.51±107.60	2832.72±1376.41	38.38±4.93	24.78±1.90
	UAE	9.46±0.83	Not detectable	1162.1±71.46	2025.31±0.00	44.20±5.12	24.80±2.43

Table 4. Results of different assays for *S. cumini* seed extracts

Solvent	Method	Extraction efficiency (%)	Total flavonoid (mg/ml QE/g of dry extract)	Total phenol (mM GAE/g of dry extract)	Antioxidant capacity (mM GAE/g of dry extract	Antibacterial activity (% inhibition at 100 µg/ml) of dry extract)	
						S. epidermidis	***P. oleovorans***
Methanol	Soxhlet	30.60±2.47	15.46 ± 1.98	1308.67±36.28	800.82±9.51	33.33±4.71	0.00 ± 0.00
	MAE	16.23±0.32	19.13 ± 3.01	4679.44±472.70	2104.01±185.96	69.17±3.53	0.00 ± 0.00
	MAEC	21.00±0.30	17.46 ± 0.85	4437.56±93.67	1771.86±0.00	60.00±2.35	0.00 ± 0.00
	UAE	17.30±1.2	21.08 ± 6.88	4614.69±436.74	2552.78±116.45	70.83±1.18	0.00 ± 0.00
Ethanol	Soxhlet	40.96±1.29	Not detectable	1540.61±77.81	844.26±10.20	63.33±4.71	0.00 ± 0.00
	MAE	29.33±1.41	23.54±3.69	3431.24±179.72	1541.56±106.05	60.83±1.18	5.38 ± 0.61
	MAEC	30.93±0.80	13.28 ± 1.70	3447.57±471.83	1283.39±0.00	44.17±8.24	0.00 ± 0.00
	UAE	28.16±1.51	14.72 ± 3.28	3960.69±580.73	1685±0.00	53.33±2.35	0.77 ± 1.08

ERT could not be carried out with *S. cumini* seeds as they were available in limited amount only. Procuring new seeds would have introduced batch-to-batch variation.

Table 3. Results of different assays for *P. sylvestris* seed extracts

Solvent	Method	Extraction efficiency (%)	Total flavonoid (mg/ml QE/g of dry extract)	Total phenol (mM GAE/g of dry extract)	Antioxidant capacity (mM GAE/g of dry extract)	Antibacterial activity (% inhibition at 100 ìg/ml)	
						S. epidermidis	***P. oleovorans***
Methanol	Soxhlet	14.23±1.47	3.63 ± 1.02	3476.92±114.33	2111.30±75.01	6.98±0.00	33.34 ± 1.27
	ERT	13.80±1.35	Not detectable	6338.05±218.61	2883.16±401.99	31.4±8.21	27.03 ± 1.27
	MAE	11.50±0.95	1.26 ± 0.00	10929.14±2516.09	2933.28±0.00	53.49±6.57	25.23 ± 1.27
	MAEC	11.50±1.80	10.10 ± 2.99	6938.93±375.86	3181.09±311.79	43.03±4.93	26.58 ± 1.90
	UAE	10.75±0.82	Not detectable	12486.57±289.48	3650.99±759.31	43.03±4.93	17.12 ± 2.54
Ethanol	Soxhlet	12.25±0.95	Not detectable	3466.96±397.54	1290.24±86.88	56.98±1.64	25.23 ± 10.19
	ERT	10.20±0.26	6.01 ± 0.45	4349.97±0.00	1736.22±155.40	62.79±6.58	19.37 ± 0.63
	MAE	7.33±0.51	Not detectable	4173.07±281.03	615.37±0.00	40.70±1.64	25.23 ± 5.09
	MAEC	7.50±0.40	5.72 ± 0.21	20977.36±3903.48	3221.65±255.95	41.87±0.00	18.47 ± 0.82
	UAE	9.30±1.90	4.49 ± 0.27	9995.30±3634.84	2967.11±265.57	29.07±4.92	13.97 ± 4.45

Table 2. Results of different assays for *M. zapota* seed extracts

Solvent	Method	Extraction efficiency (%)	Total flavonoid (mg/ml QE/g of dry extract)	Total phenol (mM GAE/g of dry extract)	Antioxidant capacity(mM GAE/g of dry extract)	Antibacterial activity (% inhibition at 100 µg/ml)	
						S. epidermidis	*P. oleovorans*
Water	Soxhlet	11.61±1.07	Not detectable	754.71±97.02	1802.55±0.00	19.57 ± 5.11	33.85±2.17
	ERT	6.30±0.2	6.03 ± 1.15	355.98±11.44	493.91±17.17	50.76 ± 13.93	54.27±2.04
	MAE	8.90±0.98	Not detectable	478.02±0.00	980.69±10.91	34.79 ± 2.05	44.29±14.14
	MAEC	9.73±0.15	1.26 ± 0.00	337.5±46.18	280.63±3.85	36.05 ± 1.64	25.23±0.00
	UAE	9.96±0.92	3.15 ± 0.19	290.32±0.00	2411.56±682.09	48.55 ± 1.02	36.16±1.08
Methanol	Soxhlet	11.95±0.35	19.32 ± 0.39	820.88±104.76	1181.94±143.68	32.5 ± 3.53	3.08±4.35
	ERT	10.93±0.55	6.07 ± 0.00	227.99±16.97	677.31±82.96	34.09 ± 1.06	22.86±0.00
	MAE	7.16±0.66	2.71 ± 0.00	812.73±6.72	1209.52±40.40	34.85 ± 8.57	0.00±0.00
	MAEC	7.40±0.10	4.28 ± 3.08	769.46±148.57	976.18±303.04	30.24 ± 6.57	24.33±0.00
	UAE	5.96±0.32	Not detectable	432.09±67.89	1206.88±26.54	43.48 ± 2.05	35.71±8.08
Ethanol	Soxhlet	8.43±0.46	1.56±0.098	3975.38±374.79	7345.75±222.41	40.58±2.04	39.23±3.25
	ERT	7.20±0.43	7.58±0.00	96.52±5.45	571.54±120.15	34.09±5.35	47.15±2.02
	MAE	6.50±0.95	2.17±0.74	618.9±20.67	751.20±151.76	48.48±2.14	45.00±3.03
	MAEC	7.26±0.25	9.30±0.28	2274.36±229.74	990.92±19.19	0.00±0.00	10.63±7.89
	UAE	8.00±0.65	2.23±0.09	316.35±10.91	789.40±150.40	39.13±4.09	57.86±11.11

Table 5. Results of different assays for *T. indica* seed extracts

Solvent	Method	Extraction efficiency (%)	Total flavonoid (mg/ml QE/g of dry extract)	Total phenol (mM GAE/g of dry extract)	Antioxidant capacity (mM GAE/g of dry extract)	Antibacterial activity (% inhibition at 100 µg/ml)	
						S. epidermidis	*P. oleovorans*
Water	Soxhlet	40.74±4.13	13.85±2.92	1139.39±147.26	1597.22±128.81	23.33±2.35	0.00±0.00
	ERT	14.66±0.60	Not detectable	999.99±188.56	433.32±47.142	34.89±9.72	33.34±0.00
	MAE	17.76±0.80	2.49±0.00	1219.10±139.79	476.43±162.63	32.61±1.02	30.77±4.34
	MAEC	12.46±1.20	Not done[#]	Not done[#]	Not done[#]	38.39±1.26	27.48±0.63
	UAE	7.13±0.40	4.72±0.00	498.82±8.30	249.99±29.11	33.3±6.20	10.77±2.17
Methanol	Soxhlet	6.95±0.05	13.85±2.92	10037.64±567.81	9006.27±1494.26	9.85±1.06	0.00±0.00
	ERT	5.20±0.43	36.86±14.99	1429.33±2.53	3108.10±197.80	39.4±4.28	27.15±0.00
	MAE	3.60±0.17	16.52±3.69	6327.16±122.57	3260.82±0.00	61.59±1.02	27.11±0.83
	MAEC	3.66±0.25	5.02±0.37	16328.24±673.30	2503.78±259.09	43.75±1.26	31.54±0.00
	UAE	2.63±0.15	11.75±2.00	8581.04±391.11	2807.00±992.43	25.76±8.57	36.43±3.03

[#]Water extract of *T. indica* prepared through MAEC formed a shiny film after evaporation and could not be dissolved in methanol while attempting reconstitution, making this particular extract unavailable for phenol, flavonoid, and antioxidant assays.

Table 6. Scoresheet for all methods with respect to different parameters

Parameter	Soxhlet	ERT	MAE	MAEC	UAE
Extraction efficiency	11	0	0	0	0
Antioxidant capacity	3	1	1	2	5
Total flavonoid	1	2	3	2	3
Total phenol	4	2	0	3	2
Antibacterial activity against *P. oleovorans*	1	3	3	0	3
Antibacterial activity against *S. epidermidis*	2	3	2	1	2

Table 7. Scoresheet for solvents with respect to different parameters

Parameter	Solvent		
	Water	Methanol	Ethanol (50%)
Extraction efficiency	2	2	1
Total flavonoid	0	5	0
Total phenol	0	3	2
Antioxidant capacity	1	3	1
Antibacterial activity against *P. oleovorans*	0	3	2
Antibacterial activity against *S. epidermidis*	2	2	1

Table 8. Scoresheet for comparison of MAE & MAEC

Method	Extraction efficiency	Total flavonoid	Total phenol	Antioxidant capacity	Antibacterial activity	
					S. epidermidis	*P. oleovorans*
MAE	1	4	5	6	8	7
MAEC	10	4	5	4	3	3

3.Results and Discussion

Results of different assays for all the seed extracts are presented through Table 1-5. Maximum value obtained in a particular assay for each extract is highlighted in bold. Based on whether the difference among results of two or more methods are statistically significant or not (results of statistical analysis for each data set not included in paper), each method was given some score. Scoresheet presented in Table 6 indicates suitability of each method for a particular purpose (e.g., flavonoid extraction, antibacterial activity, etc.). Score in Table 7 indicates suitability of each solvent used for a particular purpose. Each method (or solvent) was given a score of 1, every time it registered maximum value for a particular parameter. Scores for various methods ranged from 0-11, 11 being the maximum possible score (as total number of extracts prepared was 11). On the same line a scoresheet for comparison of both the microwave based methods (MAE and MAEC) was prepared (Table 8).

ERT, MAE, MAEC and UAE were not as good as Soxhlet with respect to extraction efficiency but these methods proved better with respect to other parameters. Extracts prepared by ERT, MAE and UAE exerted better antibacterial activity, especially against *P. oleovorans*. Increased heat exposure during MAEC and Soxhlet extraction may have led to degradation of compounds responsible for antipseudomonas activity. MAE was unable to provide good extraction efficiency but extract prepared by MAE and UAE were high in flavonoid content. Extracts prepared in methanol had flavonoids, phenols and antioxidant activity higher than those prepared in water and ethanol. Thus, it may be suggested that methanol should be preferred for extraction of antioxidant metabolites from plant seeds.

For getting high extraction efficiency and high phenol content (and antioxidant activity thereof) Soxhlet can be considered better option. Better extraction efficiency with Soxhlet method for Chamomile flowers has been reported in literature [13]. Soxhlet proved better for extraction of antioxidant compounds on more number of occasions than ERT and microwave assisted methods. Soxhlet method had been employed for extraction of phenolic antioxidants from *T. indica* seeds and pericarp [14]. A small degree of superiority of Soxhlet over microwave extraction with respect to antioxidant activity of anthraquinones from roots of *Morinda citrifolia* was reported by Hemwimon *et al.* [15]. Soxhlet was found to be superior for getting high extraction efficiency from all the plant seeds we have used, but for the extraction of flavonoids and antibacterial compounds it was found to be inferior.

MAEC was better than MAE with respect to extraction efficiency for plant seeds. Extract prepared by MAE exerted both antistaphylococcal and antipseudomonas activities on more number of occasions than those prepared by MAEC (Table 8). It may be due to possible degradation of antibacterial components in extracts due to continuous heat application during MAEC, as continuous heating can take temperature to a level higher than that achieved with intermittent cooling in MAE. MAE and MAEC both proved suitable on equal number of occasions for extraction of phenols and flavonoids. Suitability of microwave based method for flavonoid extraction from dried cell cultures of *S. medusa* was reported by Gao *et al.* [16]. However MAE was found suitable more number of times than MAEC in context of total antioxidant capacity, which again seems to be an indication of the positive role of intermittent cooling during microwave extraction. Microwave extraction with intermittent cooling had been proposed for fast extraction of plant phenolic compounds [17].

Better flavonoid extraction by microwaves than Soxhlet from *Herba Epimedii* was reported by Chen *et al.* [18]. Though MAEC yielded better extraction efficiency than MAE, the latter proved inferior to former on none of the parameters. Additionally more number of extracts prepared through MAE contained antibacterial compounds than those through MAEC. Differences in the bioactivity of extracts prepared by these two methods may be attributed to the fact that though heat enhances mass transfer during extraction (and makes the process faster), maximum temperature attained during any heat-employing method should not surpass a certain limit. Although MAEC heats the plant material for lesser duration, the maximum temperature reached during it is likely to be higher than that in MAE. Microwave extraction is one of the most advanced extraction methods, which has the potential to play a major role in extraction and analytical quantification. Advantage of microwave assisted extraction process over other processes has been reviewed by Routray and Orsat [19]. MAE when applied with methanol as the extraction solvent seems to be one of the better ways for extraction of antibacterial compounds from plant seeds.

ERT proved to be a poor method with respect to extraction efficiency and antioxidant capacity. Extracts prepared through ERT showed good antibacterial activity. This again indicates that heat may prove detrimental to antibacterial plant metabolites, as may have been the case during MAEC. On the same line Soxhlet (a heat employing method) proved no attractive option for extraction of antibacterial compounds from plant seeds.

UAE though not yielding high extraction efficiency on most occasions was found to be superior on most number of occasions with respect to total antioxidant capacity. Ultrasonication had been applied for extraction of antioxidants from grape seeds [20]. Extracts prepared through UAE exerted good antibacterial activity on more number of occasions that those prepared by Soxhlet or MEAC. This again confirms better suitability of those methods which either do not employ heat or employ it for lesser time towards retention of antibacterial activity in given plant extract.

Methanol proved most inferior with respect to no parameter evaluated. It proved better- for extraction of phenols, flavonoids, antioxidant metabolites, and antibacterial phytochemicals- than water and ethanol (50%). Better suitability of methanol (as compared to ethanol and water) for screening and isolation of antimicrobial compounds has earlier been reported by other investigators too [21, 22]. Methanolic extracts of plant leaves with antibacterial activity better than that in aqueous extracts were reported by Nair and Chanda [23]. High phenol content in methanolic extracts was reported by Kaneria *et al.*, [24]. Water was found to be least effective for extraction of phenols and flavonoids. Extracts prepared in water also exerted no notable activity against *P. oleovorans*. Hydroalcoholic extracts prepared in 50% ethanol failed to extract high quantity of flavonoids from different plant seeds.

For evaluating any specific property in plant extracts, selection of most appropriate extraction method is required because all the methods and solvents differ in mechanism of extraction from each other. Any one method cannot be said as universally applicable for extraction of all types of bioactive metabolites. In this study extraction efficiency was found to have no notable correlation with any of the parameters assayed (i.e., phenol/flavonoid content, antioxidant capacity, or antibacterial activity). This indicates that methods which are good with respect to extraction efficiency may not be equally good in terms of efficacy. Hence,

selection of method for preparation of a particular plant extract should be made in context of activity desired.

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