IJPSR (2012), Vol. 3, Issue 08 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 April, 2012; received in revised form 14 May, 2012; accepted 21 July, 2012

EVALUATION OF EXTRACTION TECHNIQUES WITH VARIOUS SOLVENTS TO DETERMINE EXTRACTION EFFICIENCY OF SELECTED MEDICINAL PLANTS

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Keywords:
Averrhoa carambola L., *Lagenaria vulgaris* Ser., *Curculigo orchioides* Gaertn., Extractive values

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ABSTRACT

Herbal drugs play an important role in health care dynamics especially in developing countries. The key step in isolation of biologically active compounds is extraction, which is expressed as total extractive yield, yields of metabolites of interest or intensity of biological activity. Extraction of phytoconstituents from plant materials has been of tremendous interest and potential. Extractive value of the crude drug also indicates the quality as well as purity of the drug. For most extractions, relatively simple techniques, such as percolation and maceration are effective and economical. Some specific applications however, require more sophisticated and costly extraction techniques using specialized equipments. It is often possible to select the specific solvent to maximize the yields on the basis of different techniques, varying in cost and level of complexity. Therefore the objective of the present study was to compare different extraction techniques with various solvents for determination of extractive values. The plants selected for the present research work were *Averrrhoa carambola* L. fruits (Oxalidaceae), *Lagenaria vulgaris* Ser. fruits (Cucurbitaceae) and *Curculigo orchioides* Gaertn. rhizomes (Hypoxidaceae). The maximum percentage of extractive value for *L. vulgaris* Ser. fruit extract was found to be 76.26±0.46 in water using reflux for extraction, whereas *A. carambola* L. fruit extract and *C. orchioides* Gaertn. rhizome extract, the maximum percentage of extractive values were found to be 88.40±0.80 and 54.00±0.80 respectively in water using ultrasound extraction method.

INTRODUCTION: One of the aims of medicinal plant research is the isolation and identification of naturally occurring substances. Chemical analysis of extracts from plant material plays a central role in development and modernization of herbal medicine [1]. Extraction forms the first step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plants [2].

The determination of extractable matter refers to the quantity of constituents in a given amount of medicinal plant material extracted with solvents; such extracted values provide an indication of the extent of polar, medium polar and non-polar components present in the medicinal plant material [3]. Solvents or the extractive agents used in the preparation of phyto-pharmaceuticals must be suitable for dissolving the important constituents and separating them.

The solubility of the drug is in large measure due to the polarity of the solvent. A general principle is like dissolves like. Thus non polar solvents will extract out non polar substances and polar material will be extracted out by polar solvents [4]. The extractive value of the crude drug determines the quality as well as purity of the drug [5].

Extraction of any crude drug with particular solvent yields a solution containing different phyto-constituents. The composition of these phyto-constituents extracted in that particular solvent depends upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of the particular drug sample.

The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional techniques require longer extraction time, thus running a severe risk of degradation for most of the phyto-constituents [6].

The demand for new extraction methods has encouraged the development of alternate extraction techniques; such as reflux and sonication. The mechanical effects of ultrasound induce a greater penetration of solvent into cellular membranes and walls, facilitating the release of contents of the cells and improve mass transfer [7].

It is often necessary to remove solvent from an extract to recover a solid mass (marc). There are several ways to do this e.g. simple distillation; Reduced-Pressure Evaporation etc. Similarly, solvent can be evaporated by placing the solution in an open container (an Erlenmeyer, evaporating dish, beaker and vial). The container is set on a heat source (boiling water bath, steam bath, hot plate, etc.) and the solvent is boiled off.

However, the problem with open-dish evaporation is that the solvent is released into the air and if the solvent is a hazardous compound, it is better to choose another method of solvent removal like use of Rota - evaporator. It is standard equipment used in modern chemistry laboratories.

The evaporation of solvent from the extract is done under reduced pressure with rotary evaporator, which can help in reducing the longer time of evaporation, degradation of thermo labile components and help in recovery of the solvent.

Averrhoa carambola L., known as carambola or star fruit is found to possess a high level of insoluble dietary fiber, alcohol insoluble solid and water insoluble solid. These fiber rich fractions might help to control post prandial serum glucose [8, 9]. Fruits are also useful in diarrhoea, vomiting, haemorrhoids, intermittent fever, scabies and general debility. Also the fruit is astringent to the bowels, allays thirst and is very much useful in the elimination of intestinal worms [10].

The ripe fruit is a good remedy for bleeding piles, particularly for internal piles. It is also useful in relieving thirst and febrile excitement. The dried fruit is given in fevers and possesses antiscorbutic properties. It is considered as one of the best Indian cooling medicines [11]. The ripe fruit is used in jelly making and provides a considerable market potential as a garnish for salads and drinks.

In western countries, the fruit is generally eaten at a ripe stage when it is yellow. However, in some Asian countries, the mature fruit is relished and consumed as fresh and in pickle preparation. The fruit is also a potential source of pectin [12].

Lagenaria vulgaris Ser. is common vegetable in India. Fruits are traditionally used for its cardio protective, cardiotonic, general tonic, diuretic, aphrodisiac, and antidote to certain poisons, alternative purgative and cooling effects. It cures pain, ulcer and fever and used for pectoral cough, asthma and other bronchial disorders-especially syrups prepared from the tender fruits [13, 14, 15]. The pulp of the fruit is considered cool, diuretic, antibilious and useful in coughs and as antidote to certain poisons [15, 16].

The fruits are reported to contain more soluble dietary fibers than insoluble cellulose fibers, soluble dietary fiber are having profound effect in lowering cholesterol, which indicates that pectin may be a predominant component of soluble dietary fibers [17].

Curculigo orhciodies Gaertn. rhizomes are sweet, cooling, diuretic, aphrodisiac, viriligenic, antipyretic and tonic and can be used against hemorrhoids, leucorrhoea, pruritis, skin diseases, bronchitis, jaundice and diarrhea [18]. It has been reported that ethanolic extract of *Curculigo orchioides* Gaertn. rhizomes possesses a hypoglycaemic activity. However, chemical and pharmacological investigations are necessary to identify the latter and to confirm its mechanism of action and its antidiabetic potential [19].

In the present study, extractive values of the selected medicinal plants were determined using various solvents according to polarity in ascending order i.e. benzene, petroleum ether, methanol, ethanol and water. For extraction, different methods used were cold extraction, Sonication (EQUITRON #8442.060.53H) and reflux; the solvents were evaporated using Rotary Vaccum Evaporator (EVATOR #EV11). This aids in selection of the most efficient technique and solvent for extraction.

MATERIAL AND METHODS: The fruits of *Averrhoa carambola* L. and *Lagenaria vulgaris* Ser.; rhizomes of *Curculigo orchioides* Gaertn. were collected from Badlapur and Karjat, (M.S., India). Herbaria were prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. The plant parts collected were washed under running tap water and blotted dry. The plant parts were then cut into small pieces and kept for drying in an oven at temperature 40±2^{o}C for five days. The dried plant parts were ground into powder, stored in airtight containers and used for further analysis. Extractive values were determined using various solvents according to polarity in ascending order i.e. benzene, petroleum ether, methanol, ethanol and water. For extraction different methods used were cold extraction, ultrasound extraction and reflux extraction. The evaporation of solvents was performed using rotary evaporator.

Extraction Procedures:

- **Cold extraction**: The coarsely powdered plant material (5 g) was extracted with 100 ml of respective solvent in a closed flask for twenty-four hours, shaking frequently during first six hours and then allowing it to stand for eighteen hours.
- **Ultrasound extraction**: The coarsely powdered plant material (5 g) was extracted with 100 ml of respective solvent in a closed flask for 30 minutes at 28±2^{o}C and 100% amplitude of output power.
- **Reflux extraction**: The coarsely powdered plant material (5 g) was extracted using reflux apparatus with 100 ml of respective solvent for 30 minutes.

After the extraction process, the filtrate was separated by filtration and the solvents were evaporated using rotary evaporator and extractive values were calculated in terms of percentage.

RESULTS AND DISCUSSION: In the present study, three different extraction methods *viz.* cold extraction, ultrasound extraction and reflux extraction were compared and according to polarity five different solvents were used for extraction.

The influence of solvent and extraction methods on the extractive values and Karl Pearsons coefficient of correlation values of *A. carambola* L. fruit powder are shown in **Table 1 and fig. 1**.

TABLE 1: EXTRACTIVE VALUES OF *AVERRHOA CARAMBOLA* L. FRUITS.

Sr. No.	Solvents	Percentage (%)		
		Cold + R.E	Sonicator + R. E	Reflux + R.E
1.	Benzene	1.86 ± 0.92	1.86 ± 0.46	1.06 ± 0.46
2.	Petroleum Ether	4.00 ± 0.80	10.40 ± 0.80	2.13 ± 0.46
3.	Methanol	51.46 ± 1.22	61.60 ± 0.80	32.00 ± 1.60
4.	Alcohol	38.93 ± 1.22	40.00 ± 1.60	20.26 ± 1.22
5.	Water	75.56 ± 1.22	88.40 ± 0.80	75.46 ± 0.46
Correlation Coefficient (r)		0.913	0.894	0.867

Key Words: R.E: Rotary Evaporator. Values are expressed as mean of three determinants ± SD

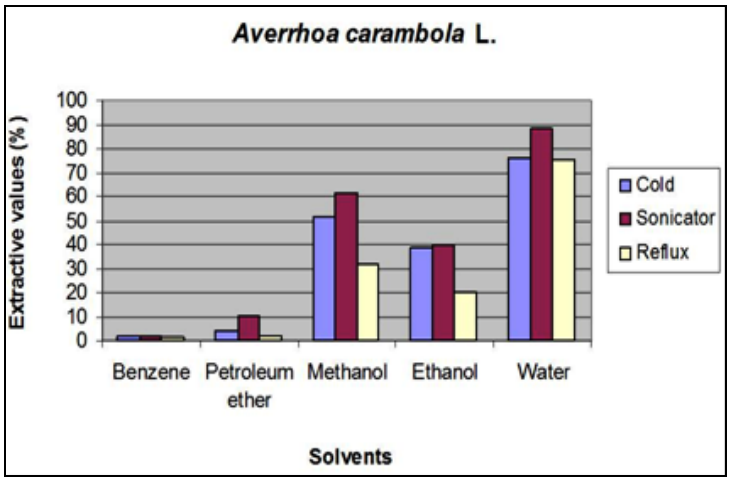

FIG. 1: EFFECT OF EXTRACTION TECHNIQUES AND SOLVENTS ON THE EXTRACTIVE VALUES OF *AVERRHOA CARAMBOLA* L.

The extractive values obtained using cold extraction method were found to be 75.56±1.22 in water followed by methanol (51.46±1.22), alcohol (38.93± 1.22), petroleum ether (4.00±0.80) and benzene (1.86±0.92), whereas the extractive values obtained using ultrasound extractions were found to be 88.40± 0.80 in water followed by methanol (61.60±0.80), alcohol (40.00±1.60), petroleum ether (10.40±0.80) and benzene (1.86±0.46). Maximum extractive value using reflux extraction was found to be 75.46 ± 0.46 in water followed by methanol (32.00±1.60), alcohol (20.26±1.22), petroleum ether (2.13±0.46) and benzene (1.06±0.46).

The influence of solvent and extraction methods on the extractive values and Karl Pearsons coefficient of correlation values of *L. vulgaris* Ser. fruit powder are tabulated in **Table 2 and fig. 2**.

The extractive value using cold extraction method were found to be 68.00±1.00 in water followed by methanol (41.60±0.80), alcohol (31.73±0.92), petroleum ether (3.20±0.80) and benzene (2.93±0.46), however the extractive values obtained using ultrasound extractions were found to be 73.00±1.02 in water followed by methanol (53.86±1.22), alcohol (35.20±0.80), petroleum ether (13.60±0.80) and benzene (2.93±0.46). The extractive value using reflux extraction was found to be 76.26 ± 0.46 in water followed by alcohol (29.60±0.80), methanol (27.73± 0.92), petroleum ether (4.00±0.80) and benzene (3.20± 0.80).

TABLE 2: EXTRACTIVE VALUES OF *LAGENARIA VULGARIS* SER. FRUITS

Sr. No.	Solvents	Percentage (%)		
		Cold + R.E	Sonicator + R. E	Reflux + R.E
1.	Benzene	2.93 ± 0.46	2.93 ± 0.46	3.20 ± 0.80
2.	Petroleum Ether	3.20 ± 0.80	13.60 ± 0.80	4.00 ± 0.80
3.	Methanol	41.60 ± 0.80	53.86 ± 1.22	27.73 ± 0.92
4.	Alcohol	31.73± 0.92	35.20 ± 0.80	29.60 ± 0.80
5.	Water	68.00 ± 1.0	73.00 ± 1.02	76.26± 0.46
Correlation Coefficient (r)		0.911	0.893	0.915

Key Words: R.E: Rotary Evaporator. Values are expressed as mean of three determinants ± SD

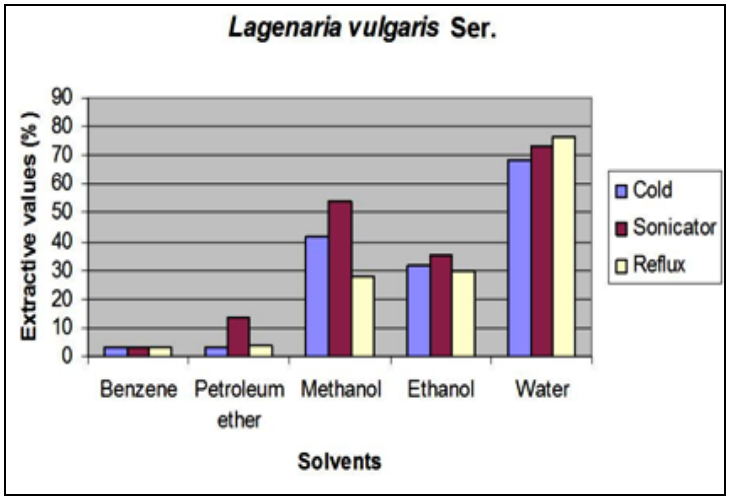

FIG. 2: EFFECT OF EXTRACTION TECHNIQUES AND SOLVENTS ON THE EXTRACTIVE VALUES OF *LAGENARIA VULGARIS* SER.

The influence of solvent and extraction methods on the extractive values and Karl Pearsons coefficient of correlation values of *C. orchioides* Gaertn. rhizomes powder are tabulated in **Table 3 and fig. 3**.

The extractive value using cold extraction method found to be 49.00±1.00 in water followed by methanol (17.60±0.80), alcohol (8.80±0.80), petroleum ether (4.26±0.92) and benzene (2.66±1.22), however, the extractive values obtained using ultrasound extractions were found to be 54.00±0.80 in water followed by petroleum ether (11.46±0.80), methanol (11.20±1.38), alcohol (6.93±0.46) and benzene (2.93±0.92).

TABLE 3: EXTRACTIVE VALUES OF *CURCULIGO ORCHIOIDES* GAERTN. RHIZOMES

Sr. No.	Solvents	Percentage (%)		
		Cold + R.E	Sonicator + R. E	Reflux + R.E
1.	Benzene	2.66 ±1.22	2.93 ± 0.92	2.93 ± 0.46
2.	Petroleum Ether	4.26 ± 0.92	11.46 ± 0.80	3.73 ± 0.92
3.	Methanol	17.60 ± 0.80	11.20 ± 1.38	8.26 ± 1.22
4.	Alcohol	8.80 ± 0.80	6.93 ± 0.46	8.00 ± 0.80
5.	Water	49.00 ± 1.00	54.00 ± 0.80	47.66 ± 0.57
Correlation Coefficient (r)		0.805	0.741	0.789

Key Words: R.E: Rotary Evaporator. Values are expressed as mean of three determinants ± SD

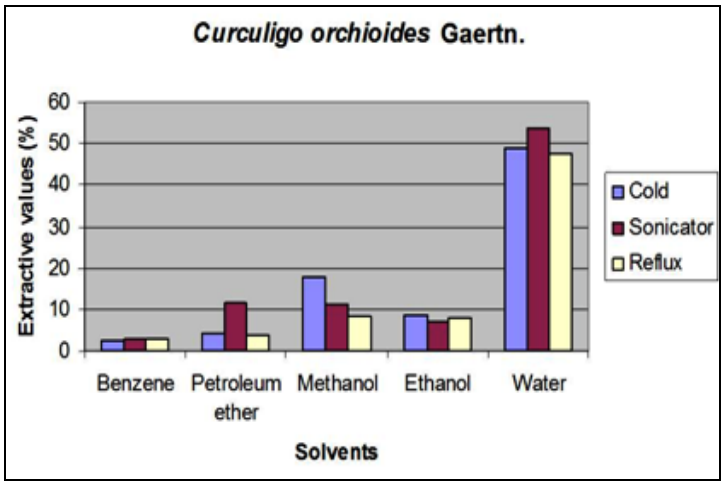

FIG. 3: EFFECT OF EXTRACTION TECHNIQUES AND SOLVENTS ON THE EXTRACTIVE VALUES OF *CURCULIGO ORCHIOIDES* GAERTN.

The extractive value using reflux extraction was found to be 47.66±0.57 in water followed by methanol (8.26±1.22), alcohol (8.00±0.80), petroleum ether (3.73±0.92) and benzene (2.93 ± 0.46).

Conventional techniques for the extraction of active constituents are time consuming, solvent use is extensive and are thermally unsafe. In order to increase the productivity efficiency, several techniques like ultrasonic waves, supercritical fluids or microwaves were associated with extraction of plant's compounds to improve the yield and quality of extracted products [20].

From the present research work, it was determined that the maximum extractive values were obtained using sonicator and reflux as methods of extraction. The maximum extraction in terms of percentage of extractive value for all the three selected plants was observed in water, revealing the presence of larger amount of water soluble constituents.

Karl Pearsons correlation coefficient (r) of the solvents used and extractive values indicated nearly perfect positive, relationship between the variables. The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractives.

The solvent used for the extraction dissolves appreciable quantities of desired constituent [21]. The selectivity of the solvent is important not only for the yield of one or more principal substances, but also for the qualitative and quantitative composition of the accompanying substances [4].

Among the three selected medicinal plants, the higher extractive values were obtained for fruits of *Averrhoa carambola* L. and *Lagenaria vulgaris* Ser. as compared to rhizomes of *Curculigo orchioides* Gaertn. The difference in the extractive values might be caused by the distinct cellular structures of and /or the different dimensions of the cells [22].

Rhizomes are largely composed of hard, mechanically resistant tissue for e.g. sclerenchyma and more amount of vascular tissues, whereas fruits comprise mainly of delicate parenchymatous cells. According to Waksmundzka-Hajnos *et al.* [22] it is possible that the destruction of the compact, hard structures and diffusion of solvents into this material requires more drastic extraction conditions than the delicate parenchyma cells.

CONCLUSION: Extractive values are useful for the evaluation of a crude drug. It is a useful tool for the estimation of constituents, soluble in that particular solvent used for extraction. From the present study, it can be concluded that aqueous extractive values of three medicinal plants selected were higher than the other solvents used. The maximum extractive values were observed using sonicator and reflux as methods for extraction. Ultrasound assisted extraction has the main advantage of working at ambient temperatures, thus avoiding the thermal overexposure, a very important asset for industry [7]. Therefore, in case of *Averrrhoa carambola* L. fruits (Oxalidaceae), *Lagenaria vulgaris* Ser. fruits (Cucurbitaceae) and *Curculigo orchioides* Gaertn. rhizomes (Hypoxidaceae), sonication and reflux extraction methods can be used as more efficient, rapid and convenient methods for the extraction of bioactive substances.

ACKNOWLEDGEMENT: Authors are thankful to UGC for sanctioning Major Research Project.

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How to cite this article:
Patil A.G., Koli SP., Patil D.A. and Phatak A.V: Evaluation of Extraction Techniques with various solvents to determine Extraction Efficiency of selected Medicinal Plants. *Int J Pharm Sci Res* 2012; Vol. 3(8): 2607-2612.