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EFFECT OF PARTICLE SIZE ON YIELD OF PLANT EXTRACT

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Keywords:

Kvatha, Plant matrix, Particle size, Extracts, Soxhlet extraction, Medicinal plants

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ABSTRACT: Plant extracts are in use in the form of decoctions and water infusions in Ayurvedic systems of medicine since ancient times. It is generally believed that particles finer in size, produce more amount of extract yield. However, there appears to be no uniformity in using powders of any particular particle size for extraction. Coarse and fine powders of different particle sizes have been observed used, by researchers and manufacturers for extraction. The object of the present study is to study the effect of the coarse and fine particles on percentage of extract yield in plants. The study is conducted on 27 medicinal plants frequently used in Ayurvedic medicine. Soxhlet extraction method using water as solvent is followed in this study. The study concludes that certain group of plants yield higher extract from coarse powders while some produce higher extract yield from fine powders. Whereas, no difference in yield of the coarse and fine powders is observed in some plants. Ayurvedic texts classify the plant material in accordance with material consistency such as very hard, moderately hard and soft, for preparation of decoctions. The present study indicates that such classification may be helpful in determining the requirement of appropriate particle size for obtaining higher extract yield.

INTRODUCTION: Use of plant extracts in medicine has been in vogue since times immemorial. *Kvatha* (decoction), *Sheeta* (cold water infusion) and *Phant* (hot water infusion), the three dosage forms among *Pancavidha Kashaya Kalpana* (five basic dosage forms), appear primitive forms of extracts found described in Ayurvedic texts. *Kvatha* (decoction) is the most popular and most frequently used independent dosage form, also used as a preliminary product to be subjected to further processing for production of dosage forms like *Ghana* (solid extract), medicated oils and ghee, *Asava* and *Arishta* (fermented medicated liquors) and many more dosage forms.

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Also known as *Shruta*, *Kvatha* is defined as the one produced by boiling the medicinal plants in water [1]. Two principal objects of preparation *Kvatha* are described by Cakrapani [2].

1. Separation of therapeutically useful plant ingredients in the solvent from the plant matrix.
2. Augmentation of therapeutic activity.

Cakrapani also states that the potency of dosage form of a drug is dependent on the quantity of therapeutically active ingredient in it. More the content of therapeutically active ingredient more will be the activity of the dosage form [1].

Thus, *Kvatha*, referred as decoction, appears to be an age-old method described in Ayurvedic classical texts for extraction of active ingredients from the dried plant material. It is also a widely used solid liquid extraction process in Ayurvedic pharma industry. As described in Ayurvedic texts, preparation of *Kvatha* involves following steps:

1. Drying, 2. Size reduction, 3. Soaking, 4. Boiling and 5. Filtration.

All these steps play significant role in determining the quantity of *Sara bhaga* i.e. active ingredient at the end of the process. The *Sara bhaga* [1] mentioned in the Ayurvedic texts, is considered as plant extract for this study. As described above, *Kvatha*, the decoction is used as an independent dosage form and also as an intermediate product to be processed further for production of another dosage form. Among the above- mentioned steps in preparation of *Kvatha,* particle size reduction of plant material is most important as the plant material can't be subjected to extraction in its natural form. Some plant materials require cutting and some require pounding for reduction in their particle size. Regarding particle size of the plant material required for preparation of decoction, laying the general guideline, describing the fineness of the particles, the word '*Anu*' meaning micro-fine, is used by Ayurvedic classics [3, 4].

Susruta and Vagbhata state that the plant parts are to be cut or broken as necessary, and then intensely pound to produce powder with fine particles. The word '*Avakuttya*' used by these authors, refers to intensive pounding. In this context, Sharangdhara uses the word '*Kshunne*' to describe the treatment given to the plant part prior to the process of preparation of *Kvatha*. The word '*Kshunne*' implies pounding, crushing and bruising of the plant part before mixing it in appropriate amount of water and boil it to produce *Kvatha,* the decoction [5]. It is generally believed that particles finer in size, will produce more amount of extract yield. However, there appears no uniformity in using powders of any particular particle size for extraction. Researchers have used very coarse (Mesh no. 20,22) [6], coarse (mesh no. 40) [7], moderately coarse (mesh no 60) [8], and fine powders (Mesh no. 120) [9]; for preparing Soxhlet extracts from stems, roots, leaves, aerial parts and whole plant. Types of particle size range for powders are shown below in **Table 1**.

However, it was also observed during our routine practice that the requirement of particle size for extraction, varies from plant to plant. This observation generated a necessity for conducting the present study.

TABLE 1: TYPES OF PARTICLE SIZE RANGES FOR POWDER

S. no.	Mesh Sieve number	Powder type	Particle size (microns)
1	2-10	Very coarse	>1000
2	20-40	Coarse	355 – 1000
3	40-80	Moderately coarse	180-355
4	80-120	Fine	125- 180
5	120-200	Very fine	90 – 125

On this background the study was designed to evaluate the amount of extract obtained from coarse and fine powders of plants frequently used in Ayurvedic medicine using Soxhlet extraction method. *Kvatha,* the decoction, is the most frequently used extraction method in Ayurvedic practice. Therefore, water was used as solvent for extraction in this study. The study was conducted on 27 frequently used medicinal plants. Two samples of each plant material 1. Coarse powder and 2. Fine powder were subjected to Soxhlet extraction to determine the effect of particle size on the extract yield. The percent yield of extract was noted and the results were compared using Student's t test using 5% level of significance.

MATERIALS AND METHODS:

Materials: 27 Medicinal plants received in the laboratory from researchers for regular analysis as listed were taken for this study **Table 2**.

TABLE 2: LIST OF MEDICINAL PLANTS INCLUDED IN THE STUDY

Sr. no.	Plant with part used	Sr. no.	Plant with part used
1	*Juniperus communis* Linn. -Roots	15	*Abutilon indicum* (Linn.) Sw.- Whole plant
2	*Ricinus communis* Linn. - Roots	16	*Solanum surattense* Burm. f. - Whole plant
3	*Pluchea lanceolata* Oliver & Hiem-Roots	17	*Glycerrhiza glabra* Linn. – Stem
4	*Cedrus deodar* Roxb. – Stem bark	18	*Rhuem emodi* Wall ex Meissn – Roots
5	*Withania somnifera* Dunal - Roots	19	*Asparagus racemosus*– Roots
6	*Randia dumetortum* Lamk - Fruit	20	*Curcuma amada* Roxb.- Rhizome
7	*Cyperus rotundus* Linn. - Roots	21	*Chlorophytum borivilianum Santapau & Fernandes-Roots*
8	*Picrorrhiza kurroa* Royle ex Benth Roots	22	*Carcumazedoria* Rosc – Rhizome
9	*Piper longum* Linn. – Fruits	23	*Terminalia chebula* Retz. – Fruits
10	*Foeniculum vulgare* Mill. - Seeds	24	*Justicia adhatoda* L. – Leaves

11	*Tribulus terristris* Linn. - Fruits	25	*Termialia arjuna* Roxb. W.& A. – Stem bark
12	*Uraria picta* Desv.- Whole plant	26	*Acorus calamus* Linn. – Roots
13	*Terminalia belerica* Gaertn (Roxb.) - Fruits	27	*Phyllanthus emblica* Gaertn. – Fruits
14	*Pueraria tuberosa* DC.-Root tuber		

Equipment: Disintegrator, Mesh Sieves no 4 and 40, Soxhlet extractor, weighing scale.

Method:

Particle size Reduction: 27 medicinal plants frequently used in Ayurvedic medicine were independently subjected to particle size reduction in disintegrator to produce powdered material. The powdered material was then passed through mesh no 40 to obtain fine powder. Thereafter, the remaining powder was sieved through mesh no. 4 to obtain very coarse powder.

Soxhlet Extraction: 50 gm of powdered plant material was placed in a thimble made of strong filter paper. The powder filled thimble was then placed in the thimble chamber of Soxhlet extractor. 100 ml extracting solvent water was placed in the round bottom flask connected to the Soxhlet thimble chamber loaded with powder filled thimble.

The bottom flask was then subjected to heating. The heating was continued for four hours to collect the extract in the bottom flask. The bottom flask containing liquid extract was then separated from the Soxhlet extractor and subjected to heating to evaporate all the water and obtain dry powder of extract.

The dried extract was measured and the percent extract value was calculated and noted. The procedure was repeated for all the 54 samples of very coarse and fine powders of 27 plants. Extract value of each sample was recorded. Percent extract value of each of the 54 samples was calculated and tabulated for further assessment. It was observed that the extract yield from coarse and fine powders varied from plant to plant. On the basis of difference in percent extract of the coarse and fine powders of samples, the plants were placed in following three groups:

Group 1: Plants producing higher extract yield from fine powders,

Group 2: Plants producing higher extract yield from coarse powders

Group 3: Plants producing almost similar extract yield from coarse and fine powders.

Accordingly, the results were tabulated in three tables.

RESULTS: Extract values of very coarse and fine powders of each of the 27 plants were compared. Unpaired students t test was applied to test the statistical significance of the difference (5% level of significance) in percent extract values of plants of each of the three groups.

Extract values of fine powders were much higher than the extract values of coarse powders of plants from group 1. The difference in the percent extract values 8.3567%+/-3.613, was statistically significant (P 0.0344) in this group **Table 3.**

TABLE 3: % EXTRACT OF PLANTS PRODUCING HIGHER YIELD FROM FINE POWDERS

Sr. no.	Plant name	% Extract	
		Very Coarse (Mesh no 4) 2 mm to 4 mm	Fine Powder (Mesh no 40) 420 microns
1	*Juniperus communis* Linn. -Roots	2.4	11.2
2	*Ricinus communis* Linn. - Roots	1.68	5.6
3	*Pluchea lanceolata* Oliver & Hiem-Roots	1.38	18.4
4	*Cedrus deodar* Roxb. – Stem bark	1.46	2.4
5	*Withania somnifera* Dunal - Roots	3.97	12.8
6	*Randia dumetortum* Lamk - Fruit	20.70	21.6
7	*Cyperus rotundus* Linn. - Roots	1.6	14
8	*Picrorrhiza kurroa* Royle ex Benth -Roots	8.8	27.2
9	*Piper longum* Linn. – Fruits	16.8	20.8
	Mean difference 8.3567+/- 3.613, t- 2.3128, P- 0.0344*		

Unpaired t test, * $p<0.05$ (statistically significant).

TABLE 4: % EXTRACT OF PLANTS PRODUCING HIGHER EXTRACT YIELD FROM COARSE POWDERS

Sr. no.	Name	% Extract	
		Very Coarse (Mesh no 4) 2 mm to 4 mm	Fine Powder (Mesh no 40) 420 microns
1	*Abutilon indicum* (Linn.) Sw.- Whole plant	9.24	4.8
2	*Solanum surattense* Burm. f. - Whole plant	10.91	6.4
3	*Glycerrhiza glabra* Linn. – Stem	27.2	14.4
4	*Rhuem emodi* Wall ex Meissn – Roots	45	18.4
5	*Asparagus racemosus*– Roots	50	20.8
6	*Curcuma amada* Roxb.- Rhizome	40	2.54
7	*Chlorophytum borivilianum* Santapau & Fernandes Roots	100	17.6
8	*Carcuma zedoria* Rosc – Rhizome	17.6	1.6
9	*Terminalia chebula* Retz. – Fruits	29.6	11.2
10	*Justicia adhatoda* L. – Leaves	25	17.6
11	*Termialia arjuna* Roxb. W.& A. – Stem bark	29.6	8.1
12	*Acorus calamus* Linn. – Roots	35	7.2
13	*Phyllanthus emblica* Gaertn. – Fruits	29.6	8.1
	Mean diff. 23/8469 +/- 6.634, t - 3.5946, P- 0.0015*		

Unpaired t test, * p<0.05 (statistically significant).

Extract values of coarse powders were much higher than the fine powders sieved through mesh no. 40 for plants soft in consistency. The difference in the extract values 23.8469 +/-6.634, was highly significant (P 0.0015) when tested using unpaired t test **Table 4**.

TABLE 5: % EXTRACT OF PLANTS PRODUCING ALMOST SIMILAR EXTRACT YIELD FROM COARSE AND FINE POWDERS

Sr. no.	Plant name	% Extract	
		Very Coarse (Mesh no 4) 2 mm to 4 mm	Fine Powder (Mesh no 40) 420 microns
1	*Foeniculum vulgare* Mill. - Seeds	60.55	55.32
2	*Tribulus terristris*Linn. - Fruits	6.35	5.6
3	*Urariapicta*Desv.- Whole plant	5.37	4
4	*Terminalia belerica*Gaertn (Roxb.) Fruits	25	20.8
5	*Pueraria tuberosa* DC. Root tuber	9.6	9.6
	Mean diff. – 3.31+/-13.493, t - 0.2453, P – 0.8124		

Unpaired t test, p<0.05 (statistically significant).

Not much difference was observed in extracts obtained from powders sieved through mesh no. 40 and extract values of coarse powders for plants in group 3. The difference in the extract values 3.3100+/-13.493, was statistically insignificant (P 0.8124) when tested using unpaired t test **Table 5.**

Extracts are used widely in medicinal preparations, nutraceuticals and other consumer product industry. Varieties of extraction techniques are used from traditional methods to advance extraction technologies. Ancient Ayurvedic classical texts mention *Kvatha* (decoction), *Phanta* (hot water infusion) and *Hima* (cold water infusion) for extraction of active ingredient mentioned as *Sara bhaga* in Ayurvedic texts. Quite a number of methods of extraction are used in pharmaceutical industry. Extraction of active compounds from plant material requires appropriate extraction methods and techniques. Soxhlet extraction method, being frequently used, is used in the present study. Therefore, although, the conclusions of this study can be generalized, the observations noted in present paper, are related to Soxhlet extraction.

Parameters like extraction solvent, temperature, extraction time (duration), solvent-to-solid ratio, and storage conditions have high influence on the amount and composition of active ingredients like antioxidants in extracts [10]. Apart from these parameters, factors like plant part and plant maturity are also significant in this respect. In addition, the particle size of the plant material plays a significant role in determining the quality and quantity of the extract. Handa S S [11] states that size reduction of plant substrates before extraction maximizes the surface area, which in turn enhances

the mass transfer of active principle from plant material to the solvent. Usually, the plant material is reduced to a size between 30 and 40 mesh. The objective for powdering the plant material is to rupture its organ, tissue and cell structures so that its medicinal ingredients are exposed to the extraction solvent. Furthermore, size reduction maximizes the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent [11]. In this respect Fongang Fotsing *et al* [12], explain that the extraction, in most cases, involves soaking the plant material in solvent for some specific time. The principle of solid–liquid extraction is that when a solid material comes in contact with the solvent, the soluble components in the solid material are dissolved in it, and move to the solvent. In solvent extraction, the mass transfer of soluble ingredients to the solvent takes place in a concentration gradient. The mass transfer rate depends on the concentration of ingredients, until equilibrium is reached. After that, there will no longer be a mass transfer from plant material to the solvent. In addition, heating the solvent can also enhance the mass transfer because of its better solubility [12].

Mass transfer of plant material to the solvent described as Gatarasatva [13] and *Muktarasata* [4] in Ayurvedic classical texts, is an indication of reaching an end point of the process of preparation of *Kvatha* (decoction). The two words '*Gatarasatva* and *Muktarasata'* indicate the status of plant material at the end of the process. Both these words indicate complete extraction of therapeutically useful part from the plant material leaving behind the marc exhausted of therapeutically active ingredients. Quantity of water in proportion to the plant material to be taken for mixing and to be retained after boiling is determined with an object to achieve *Gatarasatva* or *Muktarasata* i.e. complete extraction of active ingredient.

Regarding size reduction, Ayurvedic classics advocate use of micro-fined powder produced by intensive pounding for preparation of *Kvatha*. The results in the present study indicate that use of micro-fined powder is not always useful for obtaining expected amount of extract yield from plants. The study shows that use of coarse powder of some plants yields higher extract whereas, in case of some plants, fine powders produce higher extract yield. In case of 9 plants which were studied in Group 1, it was observed that the extract yield of fine powder (40 Mesh) of all the nine plants was significantly higher (P- 0.0344) as compared to the extract yield of coarse powder. Seven out of nine plants yielded more than double amount of extract from fine powder (Mesh 40). Only two plants *Piper longum* Linn. and *Randia dumetortum* Lamk in this group, showed lower rise in quantity of extract yield **Table 3.**

In the present study, the extract yield obtained from coarse powders of plants in Group 2 was observed to be more than double than the yield obtained from fine powders of twelve of thirteen plants, which was highly significant (P 0.0015). In case of *Chlorophytum borivilianum* Santapau & Fernandes, 100% extract yield was obtained with coarse powder, whereas with fine powder, the extract yield was only 17.2%. The only exception was that of *Justicia adhatoda* L in which the yield was found raised by 25% **Table 4.**

Five plant materials were studied in Group 3 in the present study. In this group the difference observed in the quantity of extract yield of the coarse and fine powders was statistically insignificant (P-0.8124). However, higher percentage of yield was obtained from coarse powder than fine powder. It is possible that the sample size for this group was too small to derive any specific conclusion **Table 5.**

Some studies on influence of particle size on the quantity of extract are observed conducted by researchers. One such study by Makanjuola S A[14] is noteworthy in this regard. The researcher has noted and brought to fore the following points: (1) the optimum particle size (size that maximizes antioxidant property) is solvent dependent. (2) The optimum particle size is also dependent on the antioxidant properties being measured. (3) The lowest particle size may not always give the highest antioxidant property. The researcher further states that although a reduction in particle size could always lead to increased extraction efficiency, however, a critical particle size is reached such that any further reduction in the particle size could lead to no further increase or a reduction in extraction efficiency [14]. In this context, Brewer *et al* (2014) 15, in a study comparing influence of particle size

in un-milled whole bran (coarse treatment) with same whole bran milled to medium and fine treatments; observed that the coarse treatment exhibited significantly higher antioxidant properties compared to the fine treatment; except for the ORAC value, in which the coarse extract was significantly lower [15].

Zhang *et al.* (2016) [16] reported that no significant difference was found in the total phenol content of water extract of superfine black tea powder (13.67 μm) and coarse tea powders (228.67, 161.00, 140.67, 79.07 μm). According to Vuong *et al.* [17], the extraction of catechins may be impaired when brewing very small particle sizes because these small particles may settle to the bottom and, like sand, form sediments at the bottom of the extraction container, which could reduce the flow-through of water and therefore, the tea would not effectively interact with the water.

It should also be noted that a very small powder particle size may become slimy during extraction and create difficulty during filtration. All the above-mentioned studies have reported significance of particle size of the plant material in producing adequate amount of extract.

In the present study, it is observed that some plants produce hight yield of extract with finer particles (Group 1) and some produce with coarse particles (Group 2). Whereas, in case of some plants, coarse or fine particles doesn't impact the amount of extract yield (Group 3). However, no explanation can be given for such a difference in extract yield. Description in Ayurvedic texts with regards to preparation of *Kvatha*, appears to give some lead in this regard. In this description, the authors have underlined the significance of hardness of the plant matrix during preparation of *Kvatha* (decoction) for its use in preparation of medicated oil and ghee. However, this description is related to determination of proportion of plant and water for obtaining adequate amount of active ingredient in the decoction. In this respect, Sharangdhara classifies the plant material into three types 1. *Mrudu* (soft), *Kathina* (moderately hard) and *Atyanta Kathina* (very hard) plants on the basis of hardness and compactness of the plant matrix [18]. Considering these types, the decoction to be used for preparation of medicate oil/ghee, is prepared by taking four, eight and sixteen parts of water for soft, moderately hard and very hard plant material respectively. The prescribed proportion of plant part and water is required to facilitate transfer of adequate amount of therapeutically active ingredient in the solvent, in this case water.

Thus, Sharangadhara and other authors of ancient Ayurvedic texts have considered the hardness of plant matrix for determining the proportion of water required to achieve maximum extraction of active ingredient in the decoction. However, there is no mention of requirement of specific particle size of plant material in accordance with the hardness of its matrix. Among current research studies also, none of the studies have considered the impact of particle size requirements in relation to hardness of the plant matrix. The results of present study indicate need of such consideration.

However, apparently not much uniformity in hardness of the plant matrix in intragroup plants is observed to arrive at any definitive conclusion. But majority of the plants in Group 2 appear to be relatively soft in matrix. In this group significantly higher extract yield ($p < 0.05$) is obtained from coarse powder.

Whereas, most of the plants belonging to Group 1, appear to be hard in their matrix. This group has yielded significantly higher extract from fine powder. Whereas, plants belonging to Group 3 appear to be moderately hard in their matrix. No significant difference ($p > 0.05$) was observed in extract yield from coarse and fine powders from this group of plants. Thus, the study indicates that hardness of the plant matrix appears to play significant role in determining the requirement of a particular particle size for extraction. Hardness of the plant matrix is determined by cellular infrastructure of the plant part. In this regard, Mohsenin [19] as quoted by Raza *et al* [20], states that amorphous fibrils, lignin and pectin in the cell wall are also known to enhance the strength and hardness of the stem. In this context Tanaka et al[21] also quoted by Raza et al,[19] states that Lignin or cellulose generally determines physical strength, as low content of lignin or cellulose causes a brittle culm. On the background of above information, a detailed study of plant infrastructure and its relation to hardness needs to be undertaken. Measurement

of hardness and determination of particle size in relation to the measured hardness will also prove useful for improving the extract yield. Such a study will definitely benefit the Pharma industry.

CONCLUSION: Particle size of plant material plays a significant role in determining the quantity of extract following extraction. Ayurvedic texts have noted the significance of hardness of plant matrix in determining the proportion of solvent (water) and plant material during preparation of *Kvatha*(decoction). In the present study, the need of particle size in relation to the hardness of plant matrix was studied in the context of its influence on percent extract yield. On the basis of this study, it can be concluded that requirement of particle size can't be uniform for extraction of medicinal plants. Coarse powders significantly ($P < 0.05$) produce more extract yield from plant material which are soft in matrix. Whereas, fine powders (Mesh 40) produce more amount of extract from plant material which are very hard in matrix. In case of the plant materials moderately hard in matrix, not much difference was observed in the extract yield. However, sample size being very small in this group, definitive conclusion can't be drawn.

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CONFLICTS OF INTEREST: We declare that we have no conflict of interest.

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