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RESEARCH ARTICLE

COMPARISON OF FACTORS ON THE TOTAL PYRETHRIN CONTENT EXTRACTION FROM PYRETHRUM FLOWER (*CHRYSANTHEMUM CINERARIAEFOLIUM*).

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Abstract

Extraction efficiency of insecticidal active compounds, the pyrethrins, from dried Dalmatian pyrethrum flowers (*Chrysanthemum cinerariaefolium*) was evaluated. For the study as independent factors the drying method (under shade, direct solar ray and oven temperature at 40^{o}c), the solvent type (hexane, acetone and ethanol) and the particle size (0.2, 0.5, 1 and 2 mm) were selected for the extraction of the pyrethrines. Main effects of drying method versus particle size on pyrethrum extraction of the pyrethrines in a particle size of 0.5mm has a significant difference (p=0.001) in the extract than the other three particle sizes. The interaction effect of factors in the processing methods on Pyrethrines content showed a significantly higher and statically similar pyrethrin yield was found in a particle size of 0.5mm, and solar and under shade drying technique with the respect value of pyrethrin content (1.60% and 1.56%), respectively. While, in a particle size of 1mm with the value of 1.56% and statistical uniform under shade drying system were observed best pretreatments for the extraction of the pyrethrum blossom (Table 3). The least pyrethrins content was recorded for ethanol extraction with under shade drying method in a particle size of 0.2mm with values 49.37% lower than the maximum. This result suggests that ether solar ray or under shade drying method, hexane solvent and 0.5mm particle size are more suitable technique for pyrethrum extraction process for the analysis of active ingredients in pyrethrum flowers (*Chrysanthemum cinerariaefolium*).

Introduction:-

Pyrethrum is produced from the flower part of *Chrysanthemum cinerariaefolium (*synonymously *Tanacetumcinerariaefolium*), belongs to family Asteraceae is a perennial temperate plant, bearing white flowers with yellow center. The term “pyrethrum” used to the dried and powdered flower heads of a white-flowered, daisy-like plant part belonging to the Chrysanthemum genus (Dolinšek, J *et. al.,* 2007). It is a native plant in Adriatic coastal regions where it grows in plain and lower altitudes with low temperature but it can grow in the highland region at an altitude of 2400-3000 meters above sea level and rainfall of 1000-1200 mm annually where the cool period at such altitude favor the flower initiation of pyrethrum (Duval,J. 1993; Fulton,D. *et.al*., 2001; Bisht,C. *et.al.*,

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2009). The plant is the source of important natural insecticidal ingredients known as *Pyrethrin*, which is a powerful active ingredient of pyrethrum product obtained from the flower (Klaassen, C. 1996; Bisht, C. *et.al.*, 2009).

The plant flower has a deterrent or insecticidal properties against numerous agricultural and nonagricultural insects (flies, fleas, lice, mosquito, bed bugs etc), but harmless to warm blooded mammals including human being (Purseglove, J. 1984; Stoll, G. 1998; Panda, H. 2005; Grdisa, M. *et. al*., 2009). Pyrethrum is used as an insecticide in the form of powder, spray, aerosol, coils, cream and ointment (Panda, H. 2005; Grdisa, M. *et. al*., 2009).

Pyrethrum and pyrethrins have been used as insecticides since 1800 and for decades have been the most commonly used home and garden insecticides in the US (Casida, J. and Quistad, G.1995). The increasing environmental concern, the adaptation and resistance of insects' population to synthetic insecticides are acting in favor of the natural bio-products, which do not show any of these disadvantages. Pyrethrin is composed from the extracts of pyrethrum flower with organic solvent on concentration producing dark oleoresin which contains six compounds of three groups: the Pyrethrin, Cinerin and Jasmolin in the ratio of 10:3:1 (Dolinšek, J. *et.al*., 2007, Nagar, A. *et. al*., 2015). The pyrethrin concentration in the pyrethrum extract accounts for 66 to 70% of the whole extract (Vasisht, K. 2000; Dolinšek, J. *et.al*., 2007). The different chemical compositions make the extract less adaptable to insects and its liability to sunlight, moisture and oxygen favor for its fast degradation and environment friendly. Therefore, the demand for pyrethrum flowers and other natural products with insecticidal properties are rapidly increasing in the world market due to a concern on synthetic Pyrethroid related products (Bisht, C. *et. al*., 2009; George, D. *et. al.*, 2014).

In Ethiopia the plant is cultivated in suitable agro ecology but not benefited either from the world market or the production for substitution of the pyrethrin insecticide. The quality of the products affected by different factors like plant maturity age, harvesting season, growing locations, biological origin (species, subspecies), post-harvest handlings (drying method, grinding, moisture) and processing factors (solvent, temperature, particle size) used for extraction contribute to the variation in chemical composition and contents (Dolinšek, J. *et. al*., 2007). There are different organic solvents used in extraction of pyrethrin resulting in complete different yield.

Extraction of oil, antioxidants, essential oils and others were influenced by the moisture contents of the plant material. The moisture content in the biomass play dual character reducing the intended yield to be extracted and on incurring cost due to drying in the extraction process will take more heat energy to liberate the water molecules from the cell structures.

Different extraction methods and solvents used in extraction of natural compounds from different plant materials. Hexane and petroleum ethers are among the most and widely employed organic solvents in extraction process in industries and laboratories: pyrethrin extraction (Ban, D. *et. al*., 2010 and Nagar, A. *et. al.*, 2015),on their study on comparison of the extraction method and solvents found that absence of specific solvent for extraction of pyrethrin in the types of extraction methods. The authors also concluded that petroleum ether was a good solvent in extracting with soxhlet apparatus while is preferred in ultrasound extraction method. Similarly (Nagar, A. *et. al.*, 2015) used different solvent systems of having varying degree of polarity (hexane, ethyl acetate, acetone, acetonitrile and methanol) with varying extraction techniques (percolation, sonication, soxhlet and agitation with heat), and found that each extraction techniques yielded higher pyrethrin with different solvents.

Extraction of natural products with organic solvent has a solid-liquid interaction which will be influenced by many factors such as the contact surface area, solid-liquid ratio, temperature, time and others. In the extraction of pyrethrines from the blooms of pyrethines, the sample materials were reduced in size and segregated in to four sizes. In super critical fluid CO_2 extraction of pyrethrum particle sizes and temperature are the main determining factors for efficient extraction system (Casamatta, G. *et. al.*, 1995). Lawson, O. *et.al.*, 2010 in their report on the extraction of soya seed at different thickness observed that maximum oil yield was obtained at particle size of 2mm. In this paper, the effect of solvent, drying methods and particle sizes were evaluated and discussed on the pyrethrines yield of the pyrethrum flower.

Materials and Methods:-

Site Descriptions:-

The plant material was cultivated in highlands of Bekoji area with an altitude above 2700m above sea level. The annual rain fall of the area is 1000 - 1200mm and the mean annual maximum and minimum temperatures are18.60c 7.90c, respectively.

Sample collection:-
The source of the Planting materials (pyrethrum flowers) was taken from conservation site of Kulumsa Agriculture Research Center, which are grown under experimental site. Weeding, cultivation and irrigation activities were done when required to facilitate effective establishment and growth of the pyrethrum flower.

Sample preparation:-
The pyrethrum flowers were allowed to dry uniformly to an average moisture content of 6.5 %. The selected processing parameters were drying (under shade, oven drying at 40 ^{0}C and direct sunlight drying), particle size (0.2, 0.5, 1 and 2 mm) and extraction solvents (hexane, acetone and ethanol). Sample preparations were done at Wendo-Genet Agricultural Research Center of the Ethiopian Agricultural Research Institute.

Reagents and chemicals:-
Standard pyrethrum extracts of 25% pyrethrin were purchased from Fluka chemical while acetone, ethanol and hexane solvents were purchased from local markets products of Hemedia India.

PyrethinesAnalysis:-
Two grams of powdered pyrethrum flower from each size was taken and extracted with hexane, acetone and ethanol for 7 hrs using Soxhlet apparatus boiling at atmospheric pressure. A waxy resinoid material was precipitated on cooling in a refrigerator at 4°C standing overnight. The cooled extracts were filtered with a Wattman No 2 filter paper, and from the filtrates sample solutions were prepared and the absorbance of each extract was measured by UV/Vis spectrophotometers at 227 nm which was calibrated with standard pyrethrum extracts of 25% (Dessalegne, F. *et. al.*, 2011).

Statistical Analysis:-
All samples were replicated three times and the experiment was a random analysis with full factorial of the three factors the drying method, the solvent types and the particle sizes. The analysis of variance was performed using a SAS program version 9.0 (SAS Inc) where the mean comparison was carried out at $P<0.05$ levels of significance.

Result and Discussions:-

Except the interaction effect of drying method versus particle size on pyrethrum extraction, highly significantly ($p<0.001$) difference between the main effects of processing parameters (drying method, solvent type and sieve size) and their interaction between drying method, solvent type and sieve size for pyrethrin content (Table 1).

Table 1:- Analysis of variance for effect of processing methods on pyrethrum extraction from *Chrysanthemum cinerariafolium*

Source of Variation	*DF*	Mean Square
		Total Pyrethrin content
Drying Method	2	0.2315***
Solvent Type	2	0.2791***
Sieve Size	3	0.2443***
Intra (Drying Method Sieve Size)*	6	0.0736ns
Intra (Drying Method Solvent Type*Sieve Size)*	12	0.1433***
Error	72	0.04
CV		17.35
R^2		0.67

Main effect of treatment:-
The moisture content at the extraction time for all drying methods on average were 6.5% and the mean pyrethrines content in the studied samples was 1.20% of all treatments. The conditioning of the pyrethrum flower in oven at 40 ^{0}C and under shade were higher and statistically uniform in preserving the high pyrethrines content than an open area exposed to solar ray with the values 1.25% and 1.24%, respectively (Table 2). Similar reports on drying

effects on pyrethrines were made that drying of plant material under shade or cooled room doesn't affect the pyrethrines content (Morris, S. et al 2006). Drying of pyrethrum flower in an open area (sun drying) without shade significantly affects the pyrethrines content resulting in low amount of pyrethrines. This indicates that drying of pyrethrum flower under direct sunlight affect the pyrethrines content possibly by condensing or hydrolyzing the active ingredient due to the UV radiation from the sun or enzymatically modified. Therefore, drying of pyrethrum flower for higher yield of pyrethrines can be performed either in oven around 40 ^{0}C or under shade to preserve the maximum content. Studies on the effect of drying methods on baobab leaves indicate that drying under shade maintain the food proximate and nutritional quality of the material (Abioye, V. *et. al.,* 2014).

The particle size of the material influences on the accessibility of the required components in the cellular structure of the plant. Size reduction in biomass material processing helps to increase the contact surface areas in solid-liquid process interaction by breaking of the storage structure of the cell components and exposing the site of accumulation for easy accesses. Penetration of chemicals in to a solid materials influenced by time and diffusion rate which in turn governed by surface area and cellular arrangements behavior. Of the studied four particle sizes 0.5 mm was significantly different in its high pyrethrines contents (Table 2). Various reports made on different oil seed materials processing indicate that size reduction increases the release of oil by increasing the surface area (Mustapa, A. *et. al.,* 2009, Russian, T. *et. al.,* 2007, Olaniyan, A. 2007, Meziane, S. *et. al.,* 2006, Ebewele, R. *et. al.,* 2010). The mean difference in the pyrethrines isolation from milled pyrethrum flower varied from 14 % for lowest size of 0.2 mm to 20% for largest size of 2 mm. Therefore, from this experiment realized that the optimum particle size suitable for better yield (1.34%) of pyrethrines extraction to be powdered to 0.5 mm. The particle sizes of the pyrethrum flower with 0.2, 1 and 2 mm size did not show a significant difference among themselves in the release of the pyrethrines, and statistical similar with the value of 1.20%, 1.14% and 1.13%, respectively (Table 2). In finely milled flower the active content may undergo a condensation reaction due to the heat exchange in the milling process and for large particle sizes that the solvent takes longer time to travel a long distance in both parallel and perpendicular direction of fiber which can lower the release of the active ingredients.

Table 2:- Main effect of processing methods on Pyrethines content

Factor	Treatment	Sample N	Mean*
Drying Method	Under Shade	36	1.24^{a}
	Solar	36	1.11^{b}
	Oven	36	1.25^{a}
Particle Size	0.2mm	27	1.20^{b}
	0.5mm	27	1.34^{a}
	1mm	27	1.14^{b}
	2mm	27	1.13^{b}

* Symbols of the same exponent are not significantly different.

Interaction effect of treatment:-

The interaction effect of factors in the processing methods on Pyrethines content showed a significantly difference ($p<0.0001$) in higher yielding of the active ingredient (pyrethrin content) was found in a particle size of 0.5mm with solar and under shade drying technique according to the respect value of pyrethrin content (1.60% and 1.56%), respectively. While, in a particle size of 1mm with the value of 1.56% and statistical uniform by under shade drying system were observed best pretreatments for the extraction of the pyrethrum blossom (Table 3).

Table 3:- Interaction effect of factors in the processing methods on Pyrethines content

Solvent Type	Particle Size mm	Mean*		
		Under Shade	**Solar**	**Oven Temperature**
Hexane	0.5	1.56^{a}	1.60^{a}	1.42^{abcd}
	0.2	1.34^{abcde}	$1.53^{bcdefgh}$	1.48ab
	1	1.56^{a}	1.20^{bcdefg}	1.20b^{cdefg}
	2	1.35^{abcde}	0.77^{j}	0.92^{ghij}
	0.5	$1.17^{bcdefgh}$	1.13^{defgh}	1.47^{abc}

Acetone	0.2	1.60^{a}	1.90^{bcdefg}	$1.30^{cdefghi}$
	1	1.07^{efj}	0.99^{fghij}	1.36^{abcde}
	2	0.87^{ghi}	0.89^{ghij}	1.42^{abcd}
Ethanol	0.5	1.27^{abcdef}	0.41^{abcd}	1^{fghij}
	0.2	0.81^{ij}	0.92^{ghij}	$1.60^{bcdefgh}$
	1	0.84^{hij}	0.94^{ghij}	1.12^{defghi}
	2	1.48^{ab}	1.12^{defghi}	1.36^{abcde}

* Symbols of the same exponent are not significantly different.

The pyrethrum blossom milled at 0.5mm size extracted with hexane solvent yielded more pyrethrines than the corresponding solvent types (Acetone and Ethanol). This means in extraction with reflux system of eluting mechanism hexane solvent is preferable in extracting pyrethrines from pyrethrum flower. More pyrethrines content was isolated from materials dried in solar ray and under shade samples (Table 3). Therefore, the factors (drying method, solvent type and particle size) in the interaction effect of each other have a significant effect on the pyrethrines content isolated at p=0.0008. This indicates that in extraction of pyrethrines components from the pyrethrum flower drying method, particle size and type of solvents employed in soxhlet extraction are crucial.

Conclusions:-

From different literature reviews, it was understand pyrethrum *(Chrysanthemum cinerariafolium)* plant is the source of important botanical and natural insecticidal ingredients known as *pyrethrin*, which is a powerful active ingredient obtained from daisy-like flowers.With respect to this different research investigations which were conducted on the study of agronomical traits with different agricultural aspects. Similar to this, it was essential studies the effect of factors on the processing of extraction methods. Hence, this study provided important information about the selection of factors on pyrethrum extract. Therefore, the study of pyrethrum flower with respect to pyrethrin content not only depends on agronomical traits but also, it requires the selection of appropriate drying method, type of solvent and particle sizes and other factors for extraction purposes. In this studies we conclude that, higher yield pyrethrin content can be extracted in hexane from a sample dried solar ray or under shade and from particle size of milled powder to 0.5mm.

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