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FORMULATION AND EVALUATION OF *HERACLEUM CANDOLLEANUM* GRANULES FOR DETERMINATION OF ANTIOXIDANT ACTIVITY

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Abstract:
This research work includes formulation and evaluation of herbal granules of Heracleum candolleanum Gamble along with determination of antioxidant activity. It is essential to determine its medicinal values for the development of herbal alternative to synthetic drugs. It is commonly called as Chittelam seeds which are native to Europe, Asia and widely found in Kerala. Successive Soxhlet extraction process used to extract the content. Presence of alkaloids, flavonoids, glycosides, terpenoids and volatile oils confirmed after phytochemical evaluation. Antioxidant activity of extracts were determined by using DPPH scavenging assay. Dried extract with antioxidant activity formulated into granules which poses excellent flow properties.

Keywords: *Chittelam seeds, Soxhlet extraction, Antioxidant activity, Herbal Granules.*

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INTRODUCTION:

Kerala is the major hub for medicinal plants in India. About 60-65% of plants used in Ayurvedic medicines are found in forest of Kerala. According to the World Health Organization, traditional medicine or herbal medicine is the accumulation of the knowledge, skills, and practices based on the theories, beliefs, and indigenized by different cultures, to maintain the health.

Herbal medicines are one type of dietary supplement. They sold as tablets, capsules, powders, extracts and fresh or dried plants. People use such types of medicines to try and maintain or improve their health. Practitioners of herbal medicine tend to concentrate on treating chronic conditions. The main advantage of herbal medicines is that they are derived from natural sources. Herbal medicines are easy to manufacture at cheap cost and can be available in large quantity with different varieties. From many years it has been seen that the herbal formulations are more effective for the diseases and disorder than other form. India is the main center of Ayurveda and herbal formulation

Heracleum candolleanum also found in western ghats of Kerala. It is native to Europe and Asia, commonly known as Chittelam seeds which belongs to family Apiceace/ Umbelliferae. It belongs to same group of plants such as fennel, cow parsley and ground elder which has the many primary biological activities. Local people of Kerala use it as, one of a spice, in their food. They also believed that it relieves mild stomach pain. The roots of the plant are large, rhizomatous and reddish in color. The stem is hollow and striated with bristly hairs. The flowers are white, sometimes pinkish and each has 5 petals. They are arranged in large umbels of up to 20 cm of diameter with 15 to 30 rays. The peripheral flowers having a radial symmetry. The small fruits are flattened and winged, elliptical to rounded in shape and glabrous (smooth, free from hair or down) up to 1 cm long. The seed dispersal is by wind.

Figure no. 1: *Heracleum candolleanum* plant

Figure no. 2: Collected seeds of *Heracleum candolleanum* plant and powdered form of seeds respectively

Present study includes detection of microscopical characteristics and identification of phytochemicals present in Chittelam seeds. Free radicle scavenging assay using DPPH to anticipate antioxidant activity by comparing with ascorbic acid as a standard.

MATERIALS AND METHODS:

Chemicals:

All chemicals and materials for present study, were collected in optimal form from the SRL chemicals and Astron chemicals. All the chemicals used were of analytical grade.

Plant collection and extraction:

Seeds of *Heracleum candolleanum* collected from the surrounding area of forest in Kerala during February 2021 and identified by local farmers. Dried seeds were triturated in mortar and pestle and extracted by using Soxhlet extraction process[1–4]. Different solvents like Petroleum ether, Benzene, Chloroform, Ethanol and Distilled Water used for successive extraction according to their polarity in eluotropic series.

Microscopic Evaluation:

Seeds of Chittelam were soaked into the water. T.S. was taken with the help of blade onto the glass slide. The T.S. of seed was stained with 10 ml of different reagents given in table no. 2 and different slides were prepared. Glass slide mounted on to the stage of microscope and observed under the size 10X of microscope. The observed structures were noted.[5]

Table no.1: Reagents used for staining of *Heracleum candolleanum*

Reagents	Observations	Characterization
Sudan Red III	Red Globules seen	Oil globules present
Safranin O	Pinkish red colored network of bowl shaped radially elongated cells	Lignified reticulate parenchyma of mesocarp and vascular bundle
Dil. Iodine solution	It gives Blue colored dots on presence Starch Grains	Starch Grains seen

Phytochemical screening:

The extract of seeds of *Heracleum candolleanum* collected in beaker and respective test tubes for determination of phytoconstituents like carbohydrates, alkaloids, glycosides, terpenoids, steroids and flavonoids[6,7]. The standard and test solution were taken in different solvents and presence or absence of phytoconstituents detected.

Table no.2: Procedure for identification of phytoconstituents

Phytochemical Tests	Observation	Inference
Liebermann – Burchard's test: Terpenoids	1ml of extract in which 2ml of chloroform and 2ml of concentrated sulfuric acid.	Brown ring at junction/ lower layer become yellow in color
Fehling's Test: Carbohydrate	1ml of extract in which 2ml of Fehling solution A & 2ml of Fehling solution B added heat the solution at 80°c for 1 h.	Brick Red ppt
Bradford test: Proteins	1ml of extract in which 2 ml of Bradford reagent was added.	Navy Blue color
Flavonoids	1ml of extract in which 2ml of sodium hydroxide (NaOH) solution was added intense yellow color formed and after addition of dil. HCL colorless solution	Intense yellow color disappears after addition of dil. acid
Ninhydrin test: Amino acids	1ml of extract add 4ml of 2% Ninhydrin solution heat at 80 °c for 1 h.	Violet color
Ferric chloride test: Phenols	1ml of extract in which 4ml of metabolic $FeCl_3$ (10%)	Green Color
Ferric chloride test: Tannins	1ml of extract in which 4ml of $FeCl_3$ (10%) aq.	Blue Color
Glycoside	1ml of extract in which 1ml of methanol and 3ml of NaOH (2.5N)	Yellow colour
Keller-kiliani test: Cardiac Glycoside	1ml of extract in which 1ml of glacial acetic acid and 1ml of 1% ferric chloride aqueous solution and 1ml of sulfuric acid.	Brown ring at junction
Borntrager's test: Anthraquinone Glycoside	1ml of extract in which 1ml of diethyl ether and 3ml of 25% ammonia solution.	Cherry red color

Extraction of volatile oil:

Volatile oil extraction is done using clevenger apparatus which works on the principal of hydrodistillation. It is a conventional process which uses water as a solvent for extraction of volatile oils and some bio-actives like flavonoids. It comprises of long tube, condenser, heating mantle and distillation flask. At the end of the long tube vapor is condensed and recovered. 20gm of powdered drug is taken with 250 ml of water in a distillation flask. Few pieces of porcelain are added to avoid bumping. Heat is provided with heating mantle. Mixture of water and oil vapours is condensed. This condensed vapour flows into separator where oil is separated and collected[8].

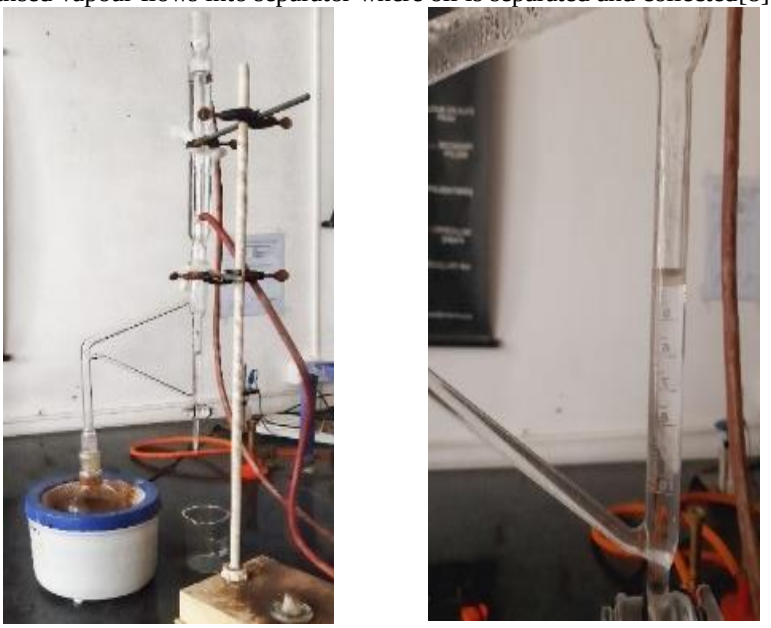


Figure no. 3: Volatile oil extraction performed by using Clevenger apparatus

In vitro Antioxidant activity:

DPPH scavenging assay

The antioxidant activity determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH)[9]. 0.05 gm of 0.2mM DPPH taken and diluted into100 ml of methanol. Flask covered with foil and kept in a dark place. Ethanolic solution of plant extract prepared and stored for 15 min. Then different concentration 100µl, 200µl, 400µl, 800µl, 1000µl of methanolic solution of extract taken into volumetric flask and made it up to 10 ml by adding methanol. From sample solution 1 ml taken and added to 3ml of DPPH solution. This diluted solution wrapped in a foil and incubated at room temperature for 30 min at dark place. After 30 min activity and absorbance of each sample solution determined at 517nm under the UV spectrophotometer.

Then percentage inhibition activity or percentage of DPPH Radical Scavenging activity was calculated by using following equation[10]

$$\% IC50 = \{(A_0 - A_1)/A_0\} X\ 100$$

Where, A_0 is absorbance of the control

A_1 is absorbance of extract/ standard.

Formulation of herbal granules:

Herbal granules prepared by using wet granulation method. After comparing different batches with different binders and disintegrants final batch used with the ingredients is Chittelam extract (active ingredient) 2.5g, starch 0.034g, Lactose as diluent 0.65g, Magnesium stearate 0.025g, Methyl paraben 0.01g[11], Talc 0.025g [12], Propylene glycol quantity sufficient as binder [13,14]. Extract dried in an oven. All dry ingredients weighed accordingly and passed through the both sieve no. 44 and 88. Powdered ingredients taken into mortar and pestle and mixed. Propylene glycol added to form a wet mass. Mixture of all ingredients and drug which formed a wet mass passed through the sieve no. 12. All formed granules are weighed and dried at 60^0C. Weighed Magnesium stearate and talc added in the formulation after drying the granules and evaluation parameters[15].

Figure no. 4: Herbal granules of *Heracleum candolleanum*

Evaluation of granules:

1. **Angle of repose:**
   It is a parameter commonly used for the evaluation interparticle force.

   The funnel technique was employed to measure angle of repose. On a flat surface graph paper placed above which funnel secured with its tip at a certain height. Granules passed through the funnel until apex of conical pile touches the tip of funnel. Radius of pile measured and angle of repose were calculated by following:

   $\tan \theta = h / r$

   h = height of heap of pile

   r = radius of base of pile

Table no. 3: Angle of repose values

Angle of Repose	Flow properties
25-30	Excellent
31-35	Good
36-40	Fair
41-45	Passable
46-55	Poor
56-65	Very Poor
>65	Very Very poor

2. **Bulk density:**
   It is defined as total volume occupied by entire powder mass. It can be determined by placing previously sieved powder bulk into a graduated cylinder and measuring the volume in milliliters. Division of original weight and attended volume gives idea about bulk density. It is obtained by measuring the volume of known mass of powder that passed through the screen.

   Granules introduced into the dry 100 ml cylinder without tapping. Volume was read. The bulk density was calculated by following formula:

   Bulk density = Mass/volume

3. **Tapped density:**
   It is determined by placing graduated cylinder containing known weight of sample on tapped density apparatus and is operated for the fixed number of taps until a constant volume is attained. Ratio of the mount of substance taken to the final constant volume gives idea

   Granules introduced into dry 100 ml cylinder by gradually tapping cylinder. Tapped Density calculated by following formula:

   Tapped density = Mass/volume

4. **Carr's compressibility index:**
   Compressibility of a powder can be defined as the ability to decrease in volume under pressure and compact ability as the ability of the powdered material to be compressed into a tablet of specified tensile strength. It can be used to predict the flow properties based on density measurement.

   It measured by following formula:

   Carr's index= Tapped density -Bulk density/ Tapped density

5. **Hausner's ratio:**
   Hausner's ratio = Tapped density / Bulk density

   :Carr's index and Hausner's ratio shown in following table.

Table no. 4: Compressibility values and Hausner's ratio values

% Carr's index	Flow character	Hausner's Ratio
<10	Excellent	1-1.11
15-16	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very Poor	1.46-1.59
>38	Very Very Poor	>16

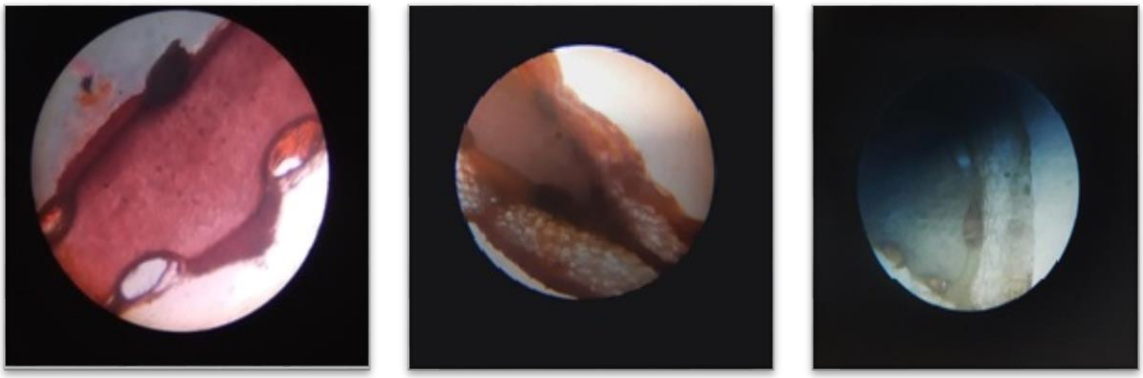
6. Disintegration time:

Granules taken and placed in meshed test tubes so that they dipped in test media which is maintained at 37^0c. They held in basket which is connected to a reciprocating shaft. The shaft allowed to move in up and down motion. Observed disintegration time noted as per I.P. limits[16,17]

RESULT AND DISCUSSION:

Microscopy:

Microscopic characters determined under the 10X power of microscope and structures identified after staining process. In Sudan red reagent presence of oil globules observed while vittae and starch grains showed the characteristic presence in safranin o and diluted iodine solution respectively. Identified structures with their respective reagents are as per figure no.5.



Sudan Red III Safranin Dil. Iodine Solution

Figure no. 5: Structures of microscopic parts after staining of Chittelam seed with reagents shows presence of oil globules, Vittae and starch.

Phytochemical screening:

After successive extraction, extract collected and tested for detection of absence or presence of phytoconstituents. Phytochemical screening gives the presence of Glycosides, Terpenoids, steroids and flavonoids compounds with test solution as shown in table no. 5.

Table no. 5: Result of phytochemical screening

Test	Petroleum ether	Benzene	Chloroform	Ethanol	Distilled Water
Carbohydrate	-	-	+	+	+
Alkaloid	-	-	-	++	++
Glycoside	-	+	-	-	-
Terpenoid	-	++	-	-	-
Steroid	-	+	-	-	-
Flavonoid	-	-	-	++	+

DPPH Scavenging assay:

By using 1,1-diphenyl-2-picrylhydrazyl (DPPH) the radical scavenging activity determined. When active compound in extract reacts with DPPH it transfers electron or hydrogen atom and neutralize its free radical character. Ascorbic acid taken as a reference standard for free radical scavenger activity and test sample compared. Prepared dilute solution observed under UV spectrophotometer and absorbance detected at 517 nm. When absorbance decreases the percentage of radical scavenging activity increases.

Table no. 6: Absorbance values and percentage of radical free scavenging activity of Ethanol extract

Concentration	Absorbance	% SCV
100	0.283	63.006536
200	0.266	63.79085
400	0.243	65.751634
800	0.236	67.843137
1000	0.202	69.019608

Ethanol extract gives the percentage of inhibition when compared to standard ascorbic acid as shown in table no. 6 and 7

Table no. 7: Absorbance values and percentage of radical free scavenging activity of standard Ascorbic acid

Concentration	Absorbance	%SCV
100	0.48	44.827586
200	0.45	48.275862
400	0.39	55.172414
800	0.33	62.068966
1000	0.24	72.413793

Graph of comparison between percentage of inhibition standard ascorbic acid and ethanolic seed extract plotted against concentration.

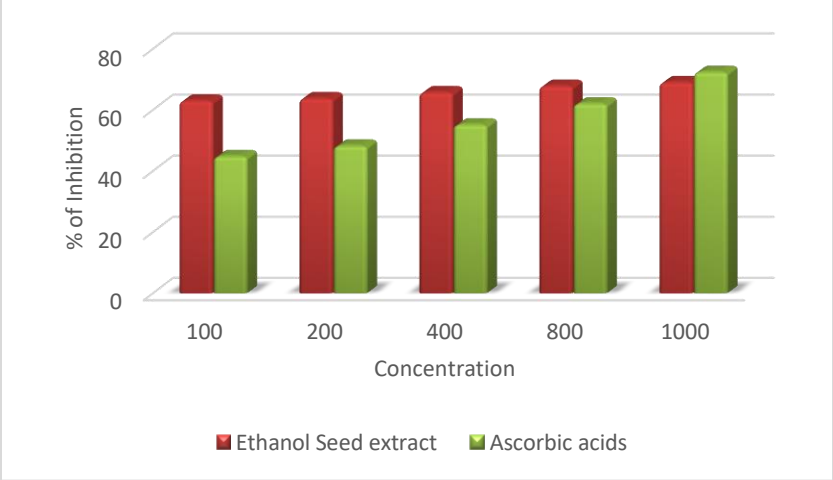


Figure no. 6: Percentage of inhibition of radical free scavenging activity by *Heracleum candolleanum* compared to ascorbic acid.

Evaluation of granules:
With Physical evaluation of granules organoleptic properties, Flow properties and Angle of repose with disintegration time recorded as per given table.

Table no. 8: Evaluation of granules

Evaluation parameters	Result
Bulk Density	0.27
Tapped Density	0.3
Hausner's Ratio	1.111
Carr's compressibility Index	10%
Angle of Repose	15.06
Disintegration time	3 min

CONCLUSION:

Present research work concluded that the *Heracleum candolleanum* i.e., Chittelam seeds contain phytoconstituents like Alkaloids, Glycosides, Terpenoid and flavonoids as it prevents free radical associate oxidative strength of any degenerative disease. After performing microscopic characterization, it is observed that the structures like oil globules, vittae and starch grains present with staining reagents Sudan red, Safranin o and dil. Iodine solution respectively. Ethanol extract of seed show DPPH scavenging activity with maximum percentage of inhibition. Extract of Chittelam seed with antioxidant activity dried and granules prepared by wet granulation method. Prepared granules evaluated and excellent flow properties with excellent angle of repose observed after the optimization study. In future Isolation and characterization of the active moiety present in different extracts of *Heracleum condolleanum* and content of volatile oil will be determined. Stability studies will be conducted for the formulation under ICH guidelines, Q1-R2.

Conflicts of interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

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