Section A-Research paper
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FORMULATION AND DEVELOPMENT OF MEDICINAL PLANT BASED DOSAGE FOR TREATMENT AGAINST INFLAMMATORY PROBLEMS

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ABSTRACT

The objective of current research work was formulation and development of medicinal plant based dosage for treatment against inflammatory problems. The physical tests conducted on the plant extract of *Arnica Montana* evaluated its nature, color, odor, and taste. The extract was in the form of coarse powder and had a yellow to orange color. It had a distinct aromatic odor, which is often described as slightly floral and herbal. In terms of taste, the extract was found to be bitter. It is important to note that these organoleptic properties were specific to the arnica extract being evaluated. The extractive values of the plant extract of *Arnica Montana* were determined for both alcoholic and aqueous solutions. The extractive value in alcohol was found to be 14.75% w/w, indicating the amount of extractable constituents present in the extract when dissolved in alcohol. On the other hand, the extractive value in aqueous solution was determined to be 21% w/w, representing the quantity of extractable constituents in the extract when dissolved in water. These values provide information about the solubility and extractability of the active constituents of *Arnica Montana* in different solvents. The total ash value of the extract was found to be 12.5% w/w, indicating the percentage of residue left after complete incineration of the extract. The water-soluble ash value, which represents the portion of ash that is soluble in water, was determined to be 8.5% w/w. Additionally, the acid-insoluble ash value, which represents the portion of ash that remains insoluble even after treatment with acid, was found to be 7.2% w/w. The phytochemical screening revealed the presence of saponins and alkaloids in the *Arnica*

Montana extract, while other tested compounds such as steroids, triterpenoids, glycosides, tannins, phenolic compounds, flavonoids, proteins, and carbohydrates were not detected. The anti-inflammatory action of extract showed potential effects in reducing edema in the carrageenan-induced rat model. The standard drug and both treated groups exhibited varying degrees of reduction in edema compared to the control group, indicating the potential anti-inflammatory activity of the formulation. In acute toxicity study no clinical signs, no deaths, remarkable body weight changes or gross necropsy was found.

Keywords: Arnica Montana, organoleptic properties, steroids, triterpenoids, glycosides, tannins, phenolic compounds, flavonoids

INTRODUCTION

Plants are extremely useful in the quest for novel medications. Around 80% of the world's population lacks access to Western medicine and is hence reliant on traditional medical methods. [1] Plant products, whether as food or botanical powders, have been utilised to heal and prevent illnesses with varied degrees of effectiveness throughout history. The widespread usage of herbal medicines and health-care preparations, such as those mentioned in ancient writings like the Vedas and made from frequently used traditional herbs and medicinal plants, may be traced back to the occurrence of natural materials having therapeutic characteristics.[2] Plants may be on the verge of making a comeback as a source of human health goods. The prospects for a return are based on the tremendous capacity of plants to create combinations of structurally varied bioactive chemicals with various and mutually potentiating therapeutic effects, as well as the unique and recently acknowledged capabilities of phytoconstituents. The chemical production of complex bioactive compounds has economic constraints. Phytoconstituents also give a more comprehensive and safer approach to illness treatment and prevention.[3]

Natural products, as the name indicates, are chemical substances formed from living organisms such as plants, animals, and insects, and natural product research focuses on their structure, synthesis, usage, and function in the organism. There is even a field of chemistry dedicated to the separation, identification, structural elucidation, and analysis of the chemical properties of chemical compounds created by living organisms. Secondary metabolites and their derivatives are the most common drugs created from natural materials, and they must now be pure and wellcharacterized molecules.[4] Phytoconstituent-rich plants exhibit a wide range of pharmacological characteristics. Flavones and flavanoids are antioxidant secondary metabolites

that are extensively dispersed. Natural components derived from plants can come from any part of the plant, including the bark, leaves, flowers, roots, fruits, and seeds.[5]

Arnica Montana, also known as wolf's bane, leopard's bane, mountain tobacco, and mountain arnica, is a moderately poisonous European flowering plant that belongs to the Asteraceae family of daisies. It is distinguished by a big yellow flower head. Another plant with the names "wolf's bane" and "leopard's bane" is aconitum, which is exceedingly toxic. Arnica Montana is used as an analgesic and anti-inflammatory herbal medication, although there is inadequate high-quality clinical evidence for these benefits, and it is poisonous when consumed or applied to wounded skin.[28]. Physiotherapy is practiced in countries such as Germany using herbal medications. The effectiveness of herbal medicine or the components included in it has been studied using preclinical and clinical models, and then formulations that meet the pharmaceutical quality of medication requirements may be conceivable.[6-7]

MATERIALS AND METHODS

Collection and Authentication of the Plant Leaves

The Arnica Montana were collected from nearby botanical garden. For the extraction, the plant flower were totally washed in distilled water, dried in the shade at room temperature for ten days, coarsely ground, and afterward went through strainer No.60.

Soxhlet Extraction

Soxhlet extraction is a common technique used for the extraction of various compounds from plant materials. Arnica Montana, commonly known as arnica, is a medicinal plant that is often used for its anti-inflammatory and analgesic properties. Soxhlet extraction can be employed to extract the active compounds from arnica. Here is a general procedure for Soxhlet extraction of Arnica Montana: Collect the Arnica Montana plant material, typically the flowers or the whole plant, and dry it thoroughly. Grinding the dried material into a fine powder can help improve the extraction efficiency. Take a clean Soxhlet apparatus, which consists of a round bottom flask, a Soxhlet extractor, a condenser, and a receiving flask. Place a small amount of an inert material (like glass wool) at the bottom of the Soxhlet extractor to prevent the plant material from directly entering the flask. Fill the Soxhlet extractor with the powdered Arnica Montana. Ensure not to pack it too tightly to allow proper solvent flow during the extraction process. Choose a suitable solvent that is capable of extracting the desired compounds from Arnica Montana. Common solvents for arnica extraction include ethanol, methanol, or a mixture of water and organic

solvents like ethanol. The choice of solvent depends on the target compounds and their solubility. Connect the Soxhlet apparatus, with the round bottom flask containing the selected solvent. Ensure that the condenser is properly attached. Start heating the round bottom flask to boil the solvent. As the solvent vaporizes, it rises to the condenser, condenses, and drips back into the Soxhlet extractor, extracting the compounds from the plant material. The extraction process continues for several hours or even overnight. The continuous cycling of the solvent helps to maximize the extraction efficiency. The collected extract may contain a high amount of solvent. To obtain a more concentrated extract, the solvent can be removed using techniques such as rotary evaporation or freeze drying. The % Yield in different solvents plant extracts were calculated by using the following formula:

*% Yield = (*Net weight of powder in gram after extraction /Total weight of powder in gram taken for extraction*) ×100*

Determination of Physical Parameters

Each plant material has its own particular physical specifications like moisture content and foreign organic materials.

Moisture content

Each medication has a shifted level of dynamic fixings, yet certain dynamic fixings are consistently present, accordingly the dampness content of a medication still up in the air on an air-dried premise. Water content in plants ought to be kept to a base to forestall breakdown and microbial pollution.

Gauged tests of 5 grams each were set in plates that were kept up with in IR dampness adjusts for 24 hours. After a particular timeframe, the weight reduction was estimated by eliminating the plate from the instrument.

Total Ash value

Subsequent to eliminating the aluminum foil and setting up the dish, it was cooked, chilled, and gauged. The dish was loaded up with 2 grams of powdered material that had been gauged. In the Muffle Furnace, the temperature was kept at around 450-500 degrees Celsius. Measure of debris assembled and gauged.

$$\% \text{ Total Ash} = \frac{\boldsymbol{weight\ of\ ash}}{\boldsymbol{weight\ of\ sample}} \text{ x } 100$$

Acid insoluble ash value

Into a little container, it poured 25 milliliters of weakened hydrochloric corrosive and washed with it. It was then separated and the extra material was washed twice with water after the measuring utencil had been bubbled for a particular measure of time. Utilizing channel paper to gather the debris, it is then positioned in an aluminum bowl and warmed to 450-500oC in the instrument. debris was gathered and gauged and corrosive insoluble debris was determined from the debris.

$$\%\text{Acid-insoluble Ash} = \frac{\boldsymbol{weight\ of\ Acid\ insoluble\ ash}}{\boldsymbol{weight\ of\ sample}} \times 100$$

Extractive values

The synthetic segments contained in the unrefined medication can be dictated by the concentrate acquired from the rough medication. Realizing that diverse synthetic segments have particular attributes and properties implies that distinctive extraction solvents are required. The solvents utilized for extraction ought to have the option to break up the required mixtures in proper sums.

Water soluble extractive value

Medications with water-dissolvable parts are estimated utilizing this measurement. In a glass container, place 5 grams of powdered plant material and 100ml of dissolvable. Cool and shake for one day. Whenever it had been separated for 24 hours it was put away in a conelike flagon. I took 25 ml of it, put it on a plate, and let it vanish on a water shower, then, at that point dried it in a 105oC broiler. In the wake of cooling, the dish was gauged, and a rate not really set in stone.

Alcohol soluble extractive value

With regards to removing optional metabolites, liquor is probably the best dissolvable. A glass holder was loaded up with 5 grams of powdered material and 100 milliliters of Solvent. The jar was cooled to room temperature and left for one day, with consistent shaking for the initial 6-8 hours of the examination. The substance was separated following 24 hours. Roughly 30 ml of the filtrate was moved to another bowl. It was important to think the filtrate and dry it in a 105°C stove. In the wake of cooling, the dish was gauged and a rate not really set in stone.

Determination of foreign matter

Pesticides, molds, creature fecal matter and other unessential materials, for example, glass and metal ought not be available in home grown prescriptions. Harmful substances incorporate pieces of the plant expected for human utilization, which should be recognized and not surpass the WHO's rules. Utilizing an amplifying focal point and an unaided eye, picked plant material was

inspected for unfamiliar materials. Gauged, the substance was determined. The way that the entirety of the plant materials were gathered by hand guaranteed that no unfamiliar material was presented, in this way the measure of unfamiliar matter was amazingly low.

Phytochemical Screening

Detection of Carbohydrate

Subsequent to dissolving 500 mg of the concentrate in five milliliters of refined water, it was then sifted. Utilizing the filtrate as a test for sugar content.

• Molisch's Test

The Molisch's reagent was added to 1 ml of filtrate in a test tube, alongside 2 ml of concentrated sulphuric corrosive. Carbonic corrosive is available at the intersection in view of a violet ring.

Molisch's reagent: To deliver Molisch's reagent, 10 grams of alpha napthol were broken down in 100 cc of 95% liquor.

• Fehling's Test

I added Fehling's answer for 1 ml of filtrate and cooked it in a water shower for 10 minutes. The presence of diminishing sugar is shown by the development of red accelerate.

Fehling's answer:

To make 500 milliliters, 34.66 grams of copper chloride were scattered in refined water.

It was delivered by dissolving in refined water, 173 gm of potassium sodium tartarate and 50 grams of sodium hydroxide.

In request to make Fehling's answer, the c) a) and b) arrangements were joined in equivalent volume.

Detection of Glycosides

0.5 gm of concentrate was hydrolyzed with 20 ml of weaken hydrochloric corrosive (0.1N) and sifted. The filtrate was utilized to test the presence glycosides.

• Modified Borntrager's

Test 01ml of filtrate was blended in with 02ml of ferric chloride arrangement at 1% in a test tube and cooked in a bubbling water shower for 10 minutes to decide the centralization of ferric chloride present. Moreover, the combination was chilled and shaken with benzene in similar extents. To eliminate benzene from the combination, a big part of its volume was treated with smelling salts. The presence of glycoside in the smelling salts layer is shown by the presence of rose pink or cherry tone.

• Killer Killiani

Test With 1 ml of frigid acidic corrosive that incorporated a hint of ferric chloride, the concentrates were shaken. Utilizing the test cylinder's sides, add 1 ml of concentrated sulphuric corrosive H2SO4. An acidic corrosive layer with a blue tone and a red fluid intersection with a red tone propose the presence of glycosides.

Detection of Alkaloids

0.5 gm of concentrate was broken up in 10 ml of weaken hydrochloric corrosive (0.1 N) and sifted. The filtrate was utilized to test the presence of alkaloids.

• Mayer's Test

Within the sight of alkaloids, filtrates were treated with Mayer's answer, bringing about the advancement of a brilliant cream-hued encourage.

Mayer's reagent:

Mercuric chloride arrangement Dissolve 1.36 grams in 60 milliliters unadulterated water.

20ml refined water with 5 grams of potassium iodide.

Combine (a) and (b) and weaken with refined water to 100 ml.

Dragendorff's Test

Filtrates were treated with Dragendroff's reagent; improvement of red shaded empower shows the presence of alkaloids.

Dragendorff's reagent:

Pour 8 grams of Bismuth Nitrate into 20 milliliters of nitric destructive and separate it completely.

50 ml pure water, 27.2 grams (gm) of potassium iodide

Combine (a) and (b) and debilitate with refined water to 100 ml.

Hager's test

As a result of using Hager's reagent on the filtrates, a yellow support molded, showing the presence of alkaloids.

Hager's reagent: Picric destructive in refined water separated in a submerged game plan.

Detection of Phytosterols and Triterpenoids

Chloroform was utilized to treat 0.5 grams of the concentrate, and the filtrate was separated. In the filtrate, phytosterols and triterpinoids were estimated.

• Salkowaski Test

Conc. H2SO4 is added to the test extricate arrangement and left to stand. The base layer turns into a rosy brown or brilliant yellow, affirming the presence of triterpenes in that arrangement.

Detection of Protein and Amino Acid

Separated water was utilized to weaken each concentrate to 100 mg. Utilizing the filtrate, scientists had the option to decide the presence of proteins and amino acids in the arrangement.

• Millon's Test

It was warmed in a water shower for 5 minutes, cooled, and afterward treated with Sodium Nitrate arrangement in a test tube with 2 ml of filtrate treated with 2 ml Million's Reagent.

The presence of proteins and amino acids is displayed by the development of a white accelerate that becomes red after warming

Millon's reagent:

Utilizing 9 ml seething nitric corrosive, break down 1g mercury. Keep a chilly blend during the interaction. When the response is finished, add refined water in an equivalent extent.

Ninhydrin Test

This was finished by adding 0.25 percent Ninhydrin reagent to two milliliters of filtrate and bubbling it for 2 minutes. The presence of amino acids is displayed by the arrangement of blue shade. Compound: 0.25 percent arrangement in butanol of Ninhydrin.

Discovery of Fixed Oils and Fats

• Oily spot test

Channel paper was set with a drop of each concentrate on it, and the dissolvable was passed on to dissipation. Foxed oil makes a sleek imprint on channel paper.

Detection of Phenolics and Tannins

100 mg of each concentrate was cooked in 1 ml of refined water and afterward separated to eliminate the pollutants prior to being utilized. A progression of tests were performed on the filtrate.

• Ferric chloride test

In a test tube, 2 ml of ferric chloride arrangement at 1% was added to 2 ml of filtrate. Within the sight of phenolic cores, a blue-dark tone is shaped.

• Lead Acetate Test

A couple of drops of lead acetic acid derivation arrangement were added to 2 ml of filtrate in a test tube. Tannins are distinguished by the presence of yellow encourage.

4.5.9 Detection of Flavonoids

• Alkaline Reagent test

A couple of drops of sodium hydroxide arrangement were applied to 100 mg of concentrate in a test tube. The presence of flavonoids is shown by the presence of a solid yellow shade that becomes dismal when weakened corrosive (HCl) is added.

Discovery of Saponin

Foam Test

For 15 minutes, 20ml of refined water was added to the concentrates before they were shaken overwhelmingly. At the point when a 1 cm layer of froth structures, Saponin is available.

Animal study

The experiment involved Wistar rats of both genders, weighing between 220 and 270 grams. The rats were housed in pairs at a temperature of 24°C and had access to abundant food and water, provided by Hindustan Lever, India. Before conducting any acute food-only tests, the animals were deprived of food for four hours. The experiments were conducted during the light phase from 08:00 to 16:00 hours.

Figure 1: Wistar rat

The animal room and polypropylene cages underwent regular and thorough cleaning to maintain a clean and well-ventilated environment. Proper aeration was ensured to promote air circulation.

Excess food and excreta were removed from the cages on a daily basis to maintain cleanliness and hygiene.

The temperature in the animal room was carefully maintained at 26 ± 2°C to provide a suitable and stable environment for the rats.

The rats were provided with daily food pellets obtained from the Poultry Research Station in Chennai. They had unlimited access to clean drinking water.

During handling, the rats were treated with gentleness and care, ensuring not to apply excessive pressure or stress on the animals.

The animals were divided into 4 groups each consisting of six animals.

Group I - Received only vehicle (Normal saline)

Group II-((Normal saline) + Diclofenac sodium (Used as a positive drug)

Group III - (Normal saline) + with Formulation with low dose

Group IV - (Normal saline) + with Formulation with high dose

Carrageenan induced rat paw edema model

Carrageenan induce rat paw edema method was used to evaluate the anti- inflammatory activity of present formulations. 0.1 ml carrageenan was induced from 1% freshly prepared suspension of carrageenan in the sub planter right hind paw of rats. This method required minimal instrument and this method is highly predictive for anti-inflammatory activity. The dose of most NSAIDs was correlated with this model of anti-inflammatory activity.

Wister rats were taken and divided into four groups with 6 rats in each. First group received 2g gel base only with no inflammation. The second group was inflamed by carrageenan and treated with low dose formulation. Third group was inflamed by carrageenan and treated with formulation, and 2nd group was inflamed carrageenan and treated with standard drug diclofenac.

Table: 1 Groups and dose in Carrageenan induced rat paw edema method

S.NO	Group	Gel composition and dose received
1.	Control group	Received 2g sod. Cmc. (gel base only)
2.	Standard drug group	Received 2g standard diclofenac
3.	Treated group low dose	Received 150 mg/kg drug extract
4.	Treated Group high dose	Received 300 mg/kg drug extract

All formulations were tested with the use of carrageen-induced paw edema for anti-inflammatory efficacy

Acute Toxicity Studies

The homogenous suspension of test drugs were prepared freshly, using 0.5% (w/v) carboxyl methyl cellulose (CMC) using a mortar and pestle. The different groups of animals(200-250 g) were administered various doses (250 g/kg p.o.) of extracts. The Animal were then critically observed for clinical symptoms, behavioral changes and mortality up to 72 h period following OECD guidelines No.423 (2001).

RESULTS AND DISCUSSION

Herbal medications often comprise a variety of pharmacologically active substances; in other cases, the exact elements that are necessary for the therapeutic action are unknown. Many herbalists feel that separated components have lesser therapeutic effects than entire plant extracts, although this is a claim that would need to be proven in each situation. Herbal medications' multi-ingredient nature can make effectiveness testing more difficult than with synthetic pharmaceuticals. One method is to consider the entire herbal extract to be the active ingredient. Extracts must be well defined to improve the repeatability of such research. This is frequently tried by standardising the extract according to a major element (e.g. a pharmacologically active ingredient or, if such an ingredient is not known, a marker suitable substance).

Numerous herbs contain compounds with anti-inflammatory properties, such as polyphenols, flavonoids, terpenoids, and alkaloids. These compounds can help reduce inflammation by inhibiting the production of inflammatory mediators like cytokines and prostaglandins. Inflammation often leads to pain and discomfort. Herbal remedies like turmeric, ginger, and boswellia have analgesic properties that can help relieve pain associated with inflammation. These herbs can inhibit pain-signaling pathways and reduce the perception of pain. Inflammation is often accompanied by oxidative stress, which can further damage tissues and exacerbate inflammation. Many herbs are rich in antioxidants that can neutralize harmful free radicals and protect cells from oxidative damage. Examples of antioxidant-rich herbs include green tea, bilberry, and ginkgo biloba. Certain herbs can inhibit enzymes involved in the inflammatory process, such as cyclooxygenase (COX) and lipoxygenase (LOX). For instance, curcumin in turmeric is known to inhibit COX-2, an enzyme involved in inflammation and pain.

Inflammation is a natural response of the body to injury, infection, or tissue damage. It is part of the immune system's defense mechanism and aims to protect the body and promote healing. However, when inflammation becomes chronic or excessive, it can contribute to various diseases and health conditions.

During an inflammatory response, the body releases chemicals such as cytokines, prostaglandins, and histamines, which help recruit immune cells to the site of inflammation. These immune cells, including white blood cells, help remove pathogens, damaged cells, and foreign substances. While acute inflammation is typically a short-term and beneficial process, chronic inflammation can be detrimental to health.

Many of today's synthetic pharmaceuticals have their origins in the plant kingdom, and herbal remedies dominated our pharmacopoeia only approximately 200 years ago. When pharmacology established itself as a dominant area of therapeutics, medical herbalism (i.e. the medicinal use of medicines that include purely plant material) saw a quick fall. Herbalism vanished off the therapeutic map in much of the English-speaking world around a century ago. Many developing countries, on the other hand, never abandoned medical herbalism (for example, Ayurvedic medicine in India, Kampo medicine in Japan, and Chinese herbalism in China), and medical herbalism coexisted with modern pharmacology in other countries, such as Germany and France, albeit at a lower level. This scenario has begun to shift again in recent years. According to a more recent US survey, 16.4% of all patients visiting an internal medicine clinic were now using herbal medications

Physical Test of Crude Drugs

The plant material were evaluated for their physio–chemical parameters and compared with the reported literature. The results compiled with the limits reported in the ayurvedic pharmacopoeia of india. The physical characteristics of Arnica montana extract, in its crude drug form, can provide valuable insights into its nature, color, odor, and taste. These properties play a significant role in identifying and characterizing the extract for various applications. The physical test results of Arnica montana extract are as follows:

Table 2: The Organoleptic properties of the plant extract were evaluated for appearance, colour and taste.

Crude drugs	Physical Test			
	Nature	Color	Odour	Taste
Arnica Montana Extract	**Coarse powder**	**Yellow to orange color**	**Distinct Aromatic odor**	**Bitter**

Table 3: Extractive Values

The Extractive Values of the plant extract were evaluated for alcoholic and aqueous solutions.

Crude drugs	Alcohol % w/w	Aqueous % w/w
Arnica montana Extract	**14.75**	**21**

Table1: Graph of the Extractive Values

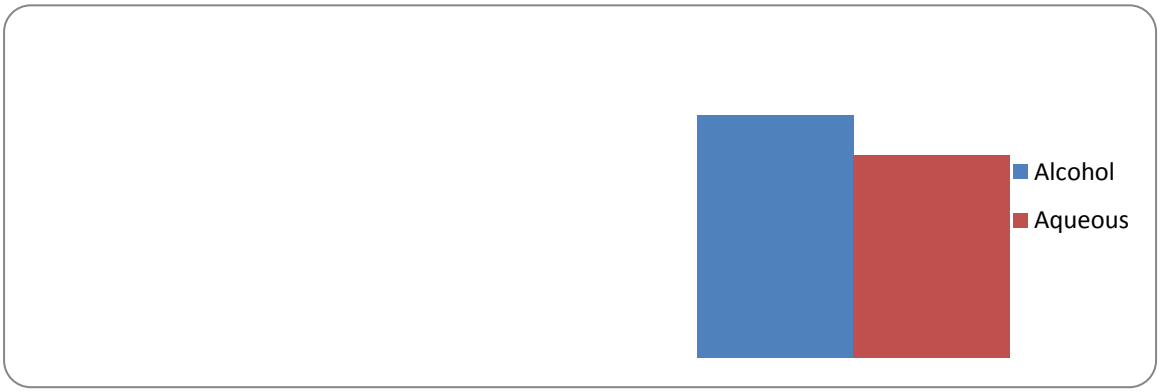


Table 4: Loss on Drying And Foreign Organic Matter (Table 5.3)

Crude drugs	Loss on drying (% w/w)*	Foreign matter (% w/w)*
Arnica Montana Extract	**7.54**	**3.20**

Figure 2: Graph of Loss on Drying and Foreign Organic Matter

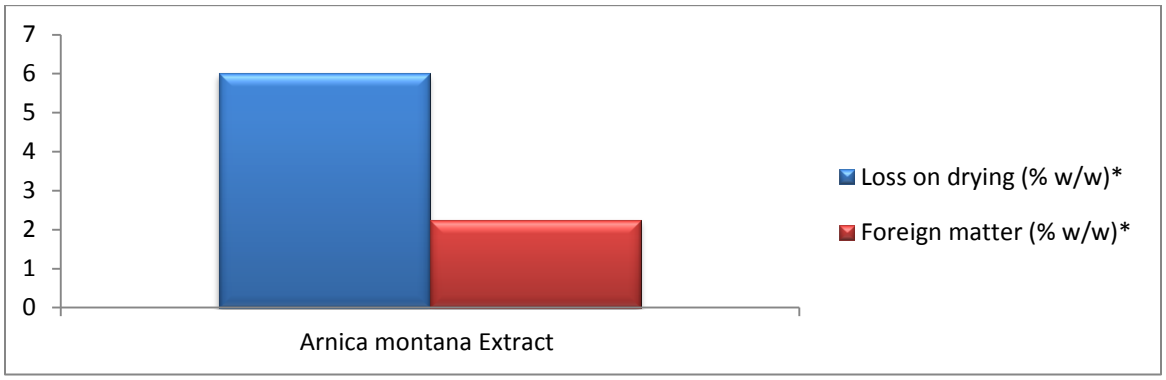
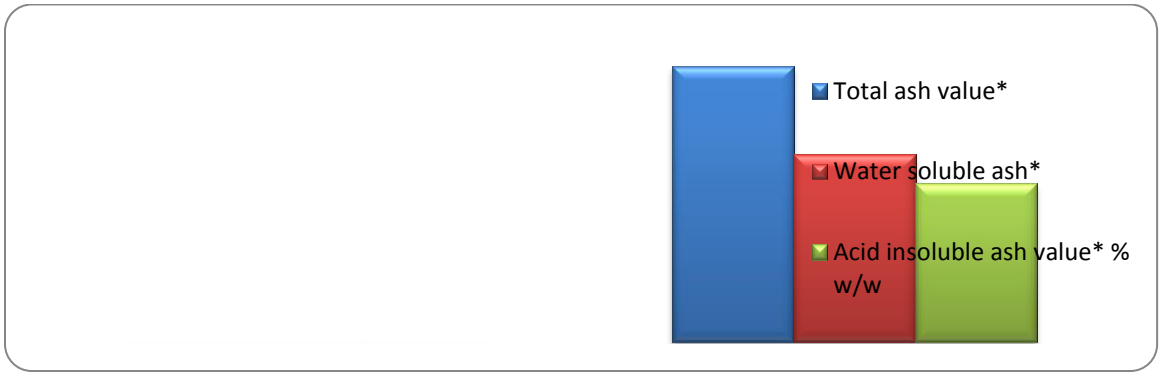


Table 5: Total Ash, Acid Insoluble Ash And Water Soluble Ash Values(TABLE 5.4)

Crude drugs	Total ash value* % w/w	Water soluble ash* % w/w	Acid insoluble ash value* % w/w
Arnica montana Extract	**12.5**	**8.5**	**7.2**

Figure 3: Graph of Total Ash, Acid Insoluble Ash and Water Soluble Ash Values:



Phytochemical Screening

The Arnica montana Extract have revealed the presence of saponins, tannins, glycosides and carbohydrates. Proteins were found to be absent in all the extracts. From this analysis, found to have more constituents in extract. The results of preliminary phytochemical screening tests of each extract presented in Table below.

S.No	Chemical Tests	Arnica montana Extract
1.	Tests for Steroids and Triterpenoids:	
	• Liebermann's Burchard Test	-
	• Salkowski Test	-
2.	Test for Saponins:	
	• Foam Test	+
3.	Tests for Alkaloids:	
	• Hager's Test	+
	• Mayer's Test	+
4.	Tests for Glycosides:	
	• Borntrager's Test	-
	• Keller Killiani Test	-
5.	Tests for Tannins and Phenolic compounds:	
	• Gelatin Test	-
	• Ferric Chloride Test	-
	• Lead Acetate Test	-
	• Dilute Nitric acid Test	-
6.	Tests for Flavonoids:	
	• Ferric chloride Test	-
	• Alkaline reagent Test	-
	• Lead acetate Test	-
7.	7. Tests for Proteins:	
	• Biuret Test	-
	• Xanthoproteic Test	-
8.	Test for Carbohydrates:	
	• Fehling Test	-

Alkaloids are commonly found to have antimicrobial properties. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds acts as primary antioxidant. Tannins are reported to prevent the growth of the many molds, yeasts, bacteria, and viruses are inhibited by tannins . Since these compounds were found to be present in the extracts, it might be responsible for the antioxidant capacity of Arnica Montana Extract. The secondary metabolites and other chemical constituents were presented in Arnica Montana Extract. Since the whole peel extracts contain the various constituents and had the number of bioactive compounds. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation. Indeed, a suitable extracting procedure should be developed and improved to recover as many antioxidants as possible before an extract rich in natural antioxidants could be further explored for possible application in health - promoting supplements for the food industry.

Table 6: Phytochemical screening for extract of Arnica montana Extract (Table 5.5)

"+" Found, "- "Not Found

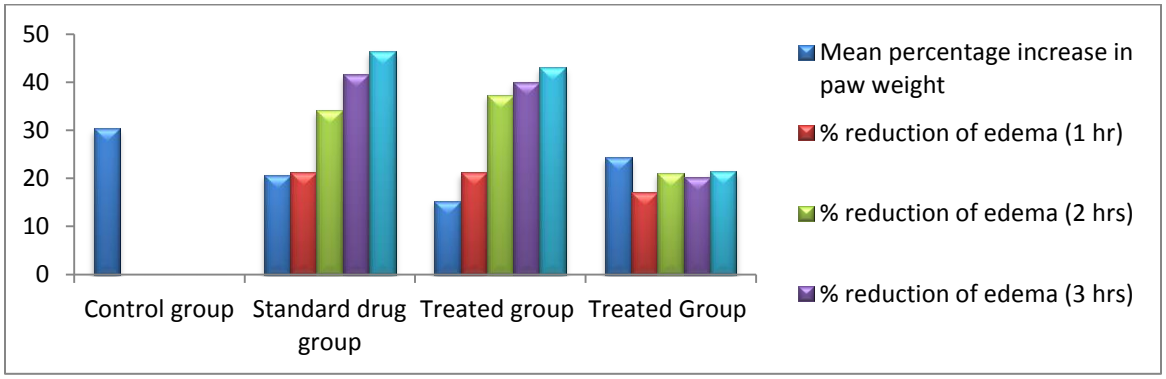
Anti-inflammatory study

Carrageenan-induced rat model was used for the anti-inflammatory investigation. Formulation 2 was determined to be appropriate for local anti-inflammatory effects as it demonstrated a 46.21% reduction in paw edoema, which was greater than the usual medication. Table Below. These findings suggest that the regular medication and the modified formulations both have anti-inflammatory effects. At all time periods, the standard medication showed a substantial reduction in edoema, whereas the treated groups also showed a significant reduction, albeit significantly less than the standard drug group. These results point to the tested formulations' potential anti-inflammatory effect in the carrageenan-induced rat paradigm.

Table 7: Carrageenan-induced rat model anti-inflammatory formulation effects

S.No	Groups	Mean percentage increase in paw weight	% reduction of edema (1 hr)	% reduction of edema (2 hrs)	% reduction of edema (3 hrs)	% reduction of edema (4 hrs)
1	Control group	30.4	0	0	0	0.0
2	Standard drug group	20.65	21.26	34.15	41.62	46.30
3	Treated group	15.30	21.25	37.30	40.10	43.01
4	Treated Group	24.30	17.10	21.12	20.14	21.40

Figure 4: The effect of formulation for anti-inflammatory study carrageenan induced rat model



Acute toxicity studies

In acute toxicity study no clinical signs, no deaths, remarkable body weight changes or gross necropsy was found which was summarized in table below.

Acute toxicity studies were performed for FM-1 and FM-2 with 150 mg/kg dose And 300 mg/kg were found to be safe.

Table 8: Acute toxicity studies

S.No	Treatment	Dose mg/kg	No. of animals	Mortality			Toxicity profile
				7 Days	14 days	21 days	
1	FM -1	150	6	0	0	0	Safe
2	FM -2	300	6	0	0	0	Safe

CONCLUSION

The physical tests conducted on the plant extract of Arnica montana evaluated its nature, color, odor, and taste. The extract was in the form of coarse powder and had a yellow to orange color. It had a distinct aromatic odor, which is often described as slightly floral and herbal. In terms of taste, the extract was found to be bitter. It is important to note that these organoleptic properties were specific to the arnica extract being evaluated. The extractive values of the plant extract of Arnica montana were determined for both alcoholic and aqueous solutions. The extractive value in alcohol was found to be 14.75% w/w, indicating the amount of extractable constituents present in the extract when dissolved in alcohol. On the other hand, the extractive value in aqueous solution was determined to be 21% w/w, representing the quantity of extractable constituents in the extract when dissolved in water. These values provide information about the solubility and extractability of the active constituents of Arnica montana in different solvents.

The ash values of the Arnica montana extract were determined to assess the inorganic content present in the extract. The total ash value of the extract was found to be 12.5% w/w, indicating the percentage of residue left after complete incineration of the extract. The water-soluble ash value, which represents the portion of ash that is soluble in water, was determined to be 8.5% w/w. Additionally, the acid-insoluble ash value, which represents the portion of ash that remains insoluble even after treatment with acid, was found to be 7.2% w/w. These values provide insights into the inorganic content and purity of the Arnica montana extract, as well as its suitability for use in various applications The phytochemical screening revealed the presence of saponins and alkaloids in the Arnica montana extract, while other tested compounds such as

steroids, triterpenoids, glycosides, tannins, phenolic compounds, flavonoids, proteins, and carbohydrates were not detected. The anti-inflammatory formulation showed potential effects in reducing edema in the carrageenan-induced rat model. The standard drug and both treated groups exhibited varying degrees of reduction in edema compared to the control group, indicating the potential anti-inflammatory activity of the formulation.

In acute toxicity study no clinical signs, no deaths, remarkable body weight changes or gross necropsy was found.

REFERENCES

1. Mukherjee P.K. Quality Control of Herbal Drugs, 2nd Ed, 2-14, Business Horizons, eBook ISBN: 9780128133989, 2007.
2. World Health Organization. Programme on Traditional Medicine. Regulatory situation of herbal medicines: a worldwide review. World Health Organization, 1998.
3. Sofowora, A., Ogunbodede, E., & Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. African journal of traditional, complementary, and alternative medicines : AJTCAM, 10(5), 210–229. https://doi.org/10.4314/ajtcam.v10i5.2
4. Chinnappan S.M., George A., Thaggikuppe P., Choudhary Y.K., Choudhary V.K., Ramani Y., Dewangan R. Nephroprotective effect of herbal extract Eurycoma longifolia on paracetamolinduced nephrotoxicity in rats. EvidBased Complement Altern Med, 2019.
5. Tungmunnithum D., Thongboonyou A., Pholboon A., Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical Aspects: An overview. Medicines, 5(3), E93, 2018.
6. Hoareau H, DaSilva EJ. Medicinal plants: a re-emerging health aid' Electronic Journal of Biotechnology. 1999;2(2) Issue of August 15, Available on line at http://www.ejb.org/content/vol2/issue2/full/2/.

7. Fitzgerald M, Heinrich M and Booker A (2020) Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. Front. Pharmacol. 10:1480.

8. Weyand, C. M., & Goronzy, J. J. (2017). Immune mechanisms in medium and large-vessel vasculitis. Nature Reviews Rheumatology, 13(12), 731–743.
9. Bajaj, J., Dave V., Sharma S., Shukla A., Chakole R.D. (2012). Pharmacognostical and phytochemical studies on achyranthes aspera. World Journal of Pharmacy and Pharmaceutical Sciences. 1(4):1316-1331.
10. Mehta, S., Garg, A., Garg, S., Kumar, M., Shukla, A. (2018). Concise report on Standardization of herbal drugs and its products. Advance Pharmaceutical Journal., 3(3):83-89.
11. Mahto, B.K., Patel R., Bapna R., Shukla A.K. (2022). Development and Standardization of a Poly Herbal Formulation. The Scientific Temper., 13(2):118-125.
12. Tiwari, R., Shukla, A.K. (2020). Plant metabolites and their role in health benefits: A brief review. Advance Pharmaceutical Journal., 5(2):47-53.
13. Pandey, P., Garg, A., Shukla, A. (2016). Preliminary phytochemical and physicochemical Investigation and thin layer chromatography of Butea Monosperma flower extract. Journal of Medical Pharmaceutical and Allied Sciences, 1-10.
14. Shahnawaz, M., Goswami, S., Shukla, A. K. (2019). Preliminary assessment of Calotropis gigantea leaves extract for in vitro antidiabetic activity. Advance Pharmaceutical Journal., 4(5):128-132.
15. 14. Pandey, P., Garg, A., Shukla, A. (2016). Preliminary phytochemical and physicochemical Investigation and thin layer chromatography of Butea Monosperma flower extract. Journal of Medical Pharmaceutical and Allied Sciences., 1-10.
16. Shukla, A., Garg, S., Garg, A., Mourya, P., Jain, C.P. (2016). Investigations on hydroalcoholic extract of Zizyphus oenoplis for analgesic and antinociceptive activity. Asian Journal of Pharmacy and Pharmacology., 2(1):15-18.

17. Gupta, M., Lodhi, S., Shukla, A. (2015). Preliminary phytochemical analysis and in vitro anti-helminthic activity of Martynia annua Linn and Permotrema reticulatum. Asian Journal of Biomaterial Research., 1(2):72-74.
18. 17. Sharma, P., Pandey, P., Gupta, R., Roshan, S., Jain, A. P., Shukla, A., Shukla, R., Garg, A., Pasi, A. (2013). Development of Quality Control Parameters for Henna Powder. Int. J. Pharm. Sci. Rev. Res., 21(1):293-295.
19. Gupta M, Lodhi S, Shukla A. Preliminary phytochemical analysis and in vitro anti-helminthic activity of Martynia annua Linn and Permotrema reticulatum Asian Journal of Biomaterial Research 2015; 1(2):72-74.
20. Bhati Pooja, A Shukla Ajay, Sharma Maya, Mourya Pramod. Hepatoprotective activity of leaves extracts of carissa carandas linn. Indo American Journal of Pharm Research.2014;4(11): 1-8.
21. Mourya Pramod, Shukla Ajay, Rai Gopal, Lodhi Santram. Hypoglycemic and hypolipidemic effects of ethanolic and aqueous extracts from Ziziphus oenoplia (L) Mill on alloxan-induced diabetic rats. Beni-Suef-University Journal of Basic and Applied Sciences, 2017; 6:1-9.
22. Gupta R, Sharma P, Garg A, Shukla A, Jain AP. 2013. Investigation of in vitro anthelmintic activity of Ficus elastica leaves. Journal of Drug Discovery and Therapeutics, 1 (5) 2013, 01-03.
23. Tiwari J, Shukla A. Investigations on Calliandra haematocephala flowers extract for in-vitro anthelmintic activity Advance Pharmaceutical Journal 2016; 1(1): 17-20.
24. Gupta M, Lodhi S, Shukla A. 2015. Preliminary phytochemical analysis and in vitro anti-helminthic activity of Martynia annua Linn and Permotrema reticulatum. Asian Journal of Biomaterial Research, 1(2):72-74.