

EVALUATION OF ARNICA MONTANA MOTHER TINCTURE EFFICACY IN PATIENTS OF RECENT INJURY AND ITS PHYTOCHEMICAL ANALYSIS

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ABSTRACT

Background: *Arnica Montana*, has been used widely used as an anti-inflammatory due to its protective action on mucus membrane. Phytochemical studies on various extracts of Arnica with varying alcoholic strength have been performed to determinethe active principles present. However, clinical trials with homoeopathic Mother tincture of Arnica on Quality of Life of patients with recent injury needs to be undertaken. **Aims and Objectives:** The aim of the study was to perform a qualitative phytochemical analysis on *Arnica montana* Mother tincture and to evaluate the efficacy of its Mother tincture on Quality of Life in patients with recent injury. **Materials and Methods:** *Arnica montana* Mother tincture was procured from a GMP certified company and it was subjected to different qualitative phytochemical. Also, a single-arm observational study was conducted at Govt. Homoeopathic Medical College and Hospital, Bhopal on 30 patients (male 21, female 9) suffering from different types of Acute upper respiratory tract infections (fulfilling the inclusion criteria) and treated by Arnica montana Q for six/seven days. The outcome measures were assessed by Quality of Life score using NRS (Numeric pain rating scale). NRS scores were measured at the baseline and after six/seven days to assess the improvement during the intervention. **Results:** The qualitative phytochemical analysis of *Arnica montana* Mother tincture revealed the presence of Terpenoids, flavonoids, Glycosides, phenolic compounds, fixed oils & fats, Phytosterols and flavonoids. Quality of Life score of the patients improved significantly after the treatment. **Conclusion:** *Arnica montana* Mother tincture contains Terpenoids, flavonoids, Glycosides, phenolic compounds, fixed oils & fats, Phytosterols and flavonoids. Arnica montana Mother tincture has a positive effect on Quality of Life of patients suffering from recent injury.

KEYWORDS: Active principles, Alkaloids, *Arnica montana*, Flavonoids, Mother tincture, NRS (Numeric pain rating scale), Phytochemistry, Quality of Life, Recent injury.

INTRODUCTION



Image 1: *Arnica montana*.

Courtesy

https://img.cosmeticsandtoiletries.com/files/base/allured/all/image/2022/09/arnica_montana_inside_ingredients_dreamstime_xxl_96662116.6324e2322f13d.png?auto=format%2Ccompress&fit=max&q=70&rect=70%2C90%2C1544%2C869&w=1200

Ancient civilization learned about the characteristics of plants, and this knowledge has been passed down through the generations to the present. The considerable interest in phytochemistry is evident from the more than 10,000 plant alkaloids that have been proved to be beneficial in the realm of medicine. Phytochemicals were extracted from the plant samples using both alcohol and aqueous methods because of solubility of phytoconstituents in these mediums. The results showed that all plant extracts included alkaloids, glycosides, carbohydrates, starches, phenolic compounds, flavonoids, proteins, pectin, mucilage, saponins, lipids, tannins, sterols and steroids.

Classical Greeks and Romans were unaware of the name "Arnica," and Early Medieval writings did not mention it either. It was reportedly originally used to name a species of magical plant in 1533 by the editor of St. Hildegard's "Physica." Dalechamps adopted this name in the 16th century, assuming it was derived from the Greek "Ptarmike." Haller and Linnaeus are responsible for the widespread use of the name "Arnica" in botany and pharmacy, linking it to the species *Arnica montana* L. (Asteraceae) starting in the 18th century.^[1]

MATERIAL AND METHODS

Phytochemical analysis of *Arnica Montana* Mother tincture

The qualitative phytochemical analysis was carried out in Pharmacy Lab at Government Homoeopathic Medical College and Hospital, AYUSH CAMPUS, Beside Kaliasot Dam, Bhopal.

Methodology

Arnica Montana Mother tincture was procured from a GMP certified company (Batch No: GM-190501) for the phytochemical screening. Reagents and chemicals were procured from a standardized laboratory.

Phytochemical tests

The sample was subjected to different qualitative phytochemical tests for the screening of the active principles.^[3]

Test for Cardiac glycosides***Keller-killani test***

Glacial acetic acid (0.4 mL) and a few drops of 5% ferric chloride solution were added to the sample solution.

Concentrated sulphuric acid (0.5 mL) was added along the side of the test tube carefully. The formation of blue colour or reddish-brown colour in the acetic acid indicates the presence of cardiac glycosides.

Test for Proteins and Amino acids***Biuret test***

5 drops of 1% copper sulphate solution and 2ml of 10% NaOH were added to 2ml of the sample. The contents were mixed thoroughly. Formation of pink to purple or violet colour indicates the presence of proteins.

Ninhydrin test

2 drops of Ninhydrin solution is added in 2mL of extract. The purple colour solution is indicate the presence of Amino acid.

Xanthoproteic test

Few drops of concentrated Nitric acid added in plant extract. The yellow colour solution indicates the presences of Protein and Amino acid.

Test for Flavonoids***Alkaline reagent test***

To the sample, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour which turns colourless after addition of few drops of dilute hydrochloric acid indicates the presence of flavonoids.

Shinoda's test

The sample was dissolved in alcohol. To that, a piece of magnesium ribbon was added followed by concentrated hydrochloric acid dropwise and subsequently heated. The appearance of a crimson red colour indicates the presence of flavonoids.

Pew's test

In few ml aqueous extract solution add 0.1gm metallic zinc and 8ml of concentrated sulphuric acid. The appearance of red colour indicates the presence of flavonoids.

Zinc-hydrochloride test

Take few ml of plant extract and add pinch of zinc dust followed by concentrated HCl along the side of test tube. The appearance of Magenta/red colour indicates the presence of flavonoids.

Test for Phenolic compounds***Ferric chloride test***

The sample was treated with dilute ferric chloride solution (5%). The appearance of Purplish red colours indicates the presence of phenolic compounds.

Test for Tannins***Braymer's test***

Few ml of filtrate mix with 3ml of distilled water and followed by few drops of 10% ferric chloride solution. Blue-green colour appearance indicates the presence of tannins.

Test for Saponins***Froth formation test***

A small quantity of the sample was diluted with 20 ml of distilled water and shaken vigorously. Formation of 1 cm layer of foam which is stable for 10 minutes indicates the presence of saponins.

Test for Triterpinoides/steroids***Salkowski reaction***

To the sample, chloroform was added followed by concentrated sulphuric acid along the sides of the tube. A golden yellow layer at bottom indicates the presence of triterpinoides/steroids.

Test for Glycosides***Aqueous NaOH test***

Alcoholic extract dissolved in 1mL of water followed by adding of few drops of aqueous NaOH solution. A yellow colour solution indicates the presence of glycosides.

Test for Phytosterols***Sulphur test***

Pinch of sulphur powder added in the extract solution. If sulphur sinks in the bottom that indicates that phytosterols are present in the extract.

Test for Quinones***Concentrated HCl test***

In plant extract add few drops of concentrated HCl. A green colour indicates the presence of quinones.

Evaluation of Mother Tincture Q on Patient of recent Injury

The study was a prospective, observational clinical type of study. Longitudinal type of study design as the same study subjects were examined after a definite time for the follow-up. The study was carried out at Government Homoeopathic Medical college and Hospital, Bhopal and Gautam Nagar Peripheral-OPD. Sample were selected as per the inclusion criteria amongst the patients visiting the Outpatient Department (OPD). The study period ranged from March 2024 to December 2024 and the study population was recruited from the OPD, at Government Homoeopathic Medical college and Hospital, Bhopal and Gautam Nagar Peripheral-OPD. Once the population was recruited, they were screened for the inclusion in the study as per the criteria. The study was targeted to be 40 which was subjected to the variation according to the availability of the subjects and time constraint. The subjects were screened from the study population as per the mentioned inclusion and exclusion criteria.

Inclusion criteria

- GMP Certified company medicine.
- Patients of all aged, sex and races.

- Patient of all socio-economic classes.
- Patient who does not have bleeding injury
- Patients agree to undergo some investigation, if required.
- Patients willing to give written consent to participate in the study.

Exclusion criteria

- Patient having any other systemic illness or any life-threatening disease.
- Any patient who have bleeding injury
- Patient not willing to participate in the study.
- Patients not willing to undergo any investigation, if so advised.
- Patients who do not undergo regular follow up or not willing to undergo further treatment for personal reasons.

Study tools and technique

Following tools and techniques were used for the study-

1. Screening form to enroll the patient for the study
2. Case recording proforma
3. Patient's interview and clinical examination
4. NRS (Numeric rating scale)

Study variables and attributes

One study variables have been taken for the statistical analysis. Quality of Life scoring of the patient through NRS was studied at the baseline and after 6th/7th day of the completion of the study.

Brief procedures

- Subjects were recruited for the study after a preliminary and detailed screening using specified screening criteria.
- Detailed and thorough case taking was performed for every patient based on the standard case taking proforma according to the format assigned by Government Homoeopathic Medical college and Hospital, Bhopal.
- Relevant clinical examination was conducted during the time of the case taking.
- An initial scoring of the Patient of recent injury of the patient was also undertaken through NRS.
- *Arnica Montana* Q (procured from the pharmacy of the study site) was prescribed to the patient orally. The dosage was given in the form of Mother tincture, twice a day, 10 drops in 45ml of water. The medicine was directed to be taken every morning and evening empty stomach for 3 days.
- The NRS score of the patients after the baseline was assessed at 6th/7th day and this was the final compilation of NRS scoring.

Outcome assessment

The response of the patient was observed and the improvement was assessed through signs and symptoms of the patient. The Quality of Life of the patients was evaluated through NRS (Numeric rating scale - English version) at the baseline and after the completion of the study.

Data collection

Data were collected from patients by using English version of the NRS for the Quality of Life scoring at the baseline and after the completion of the study. A specially designed Microsoft MS Office Excel 365 spreadsheet (master chart) was used for data extraction and was subjected to statistical analysis.

Statistical techniques and data analysis

- Data were analysed from the descriptive and inferential point of view. Baseline descriptive data (categorical and continuous) were presented in terms of absolute values, percentages, mean, standard deviation (SD) etc.
- The paired t-test** was applied for the absolute values to determine the significant changes in Patient of recent injury before and after the treatment. Value of 't' at 5% level of significance is compared.
- Microsoft Office Excel Sheet 365 for Windows was used for the analysis of data.

Ethical issues

No invasive methodology was involved and there was no therapeutic experimentation. All patients received appropriate homoeopathic treatment through oral route. No investigations were advised against the will of the patient. The proposed plan of work had been placed before the concerned Ethical committee and the permission was obtained for the same.

OBSERVATIONS AND RESULTS:

Phytochemical analysis of *Arnica montana* Mother tincture

Qualitative analysis is the first step in phytochemistry as quantitative studies are more sensitive to very small amounts of active constituents. The qualitative analysis of the sample (*Arnica montana* Q) showed the absence or presence of various phytochemicals.

S.NO.	Name of The Test	Observations	Inference
1	Salkowski's Test	Golden yellow layer at bottom	Steroids Present
2	Dragendroff's Test	No colour change	Alkaloids Absent
3	Mayer's Test	No colour change	Alkaloids Absent
4	Wagner's Test	No colour change	Alkaloids Absent
5	Aqueous NaOH Test	No colour change	Reducing sugars Absent
6	Keller-Killani Test	Colour Change to dark blue	Cardiac glycosides Present
7	Biuret Test	No colour change	Proteins and Amino acids Absent
8	Ninhydrin Test	No colour Change	Proteins and Amino acids Absent
9	Xanthoproteic Test	No colour Change	Proteins and Amino acids Absent
10	Alkaline Reagent Test	Solution become transparent	Flavonoids Present
11	Shinoda's Test	Pink Colour	Flavonoids Present
12	Pew's Test	Change to red colour	Flavonoids Present
13	Zinc-hydrichloride reduction Test	Dark pink Colour	Flavonoids Present
14	Ferric chloride Test	Purplish-red Colour	Phenolic compound Present
15	Braymer's Test	No colour change	Tannins Absent
16	Foam Test	Foam not present for 10 min.	Saponins Absent
17	Sulphur Test	Sulphur Powder sinks in Bottom	Phytosterols Present
18	Conc. HCl Test	No colour Change	Quinones Absent
19	Spot Test	Oil stain on the paper	Fixed oils and fat Present

The qualitative phytochemical analysis of *Arnica montana* Mother tincture reveals the presence of Terpenoids, flavonoids, Glycosides, phenolic compounds, fixed oils & fats, Phytosterols and flavonoids. However, Steroids, Alkaloids, Carbohydrates, Reducing sugar, Saponins, Quinones, proteins and amino acids were not detectable.

Efficacy of *Arnica montana* Q on Patient of recent injury**Study flow**

As per the pre-specified inclusion and exclusion criteria, 40 patients suffering from Patient of recent injury were screened; 10 were excluded on account of various reasons (listed in figure 2); 30 fulfilled the eligibility criteria and were enrolled. Baseline NRS value was obtained for these 30 enrolled patients. The dose of the intervention was adjusted as per the study design (Figure 1). Baseline NRS was taken into consideration. The follow up was taken after 6th/7th day with NRS. After 6th/7th day of intervention, NRS value was determined and the outcome data were recorded. During treatment, 30 patients completed the trial.

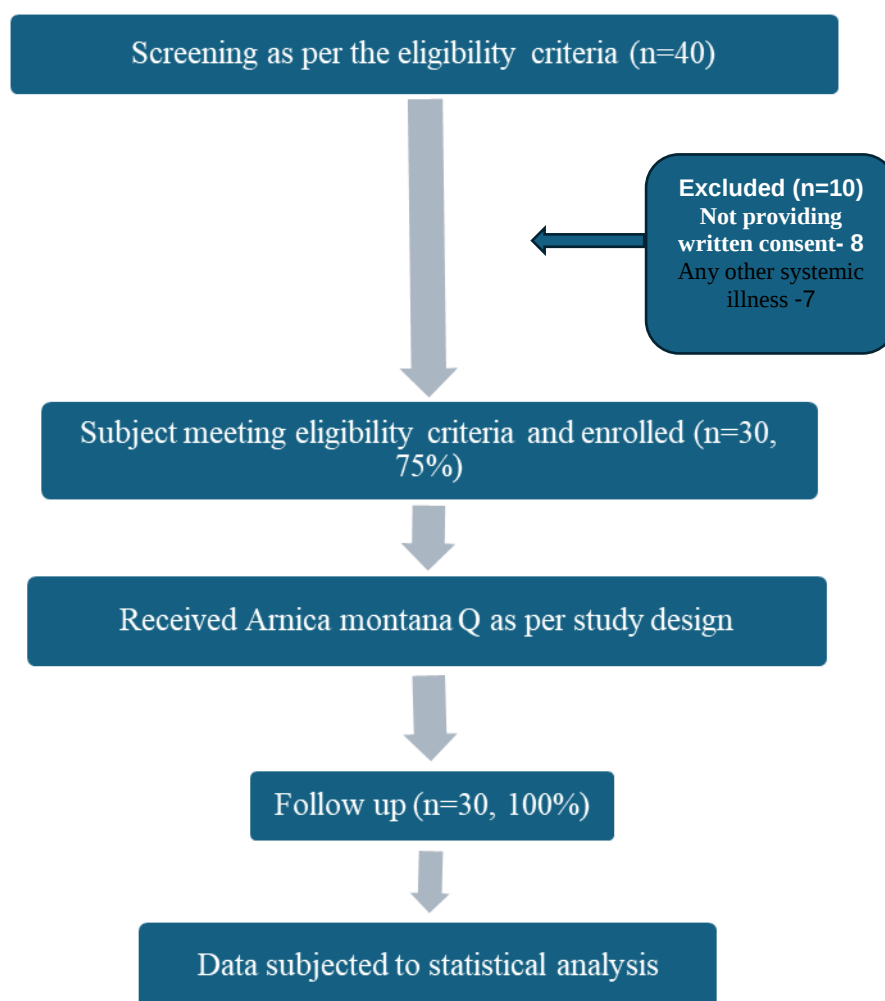


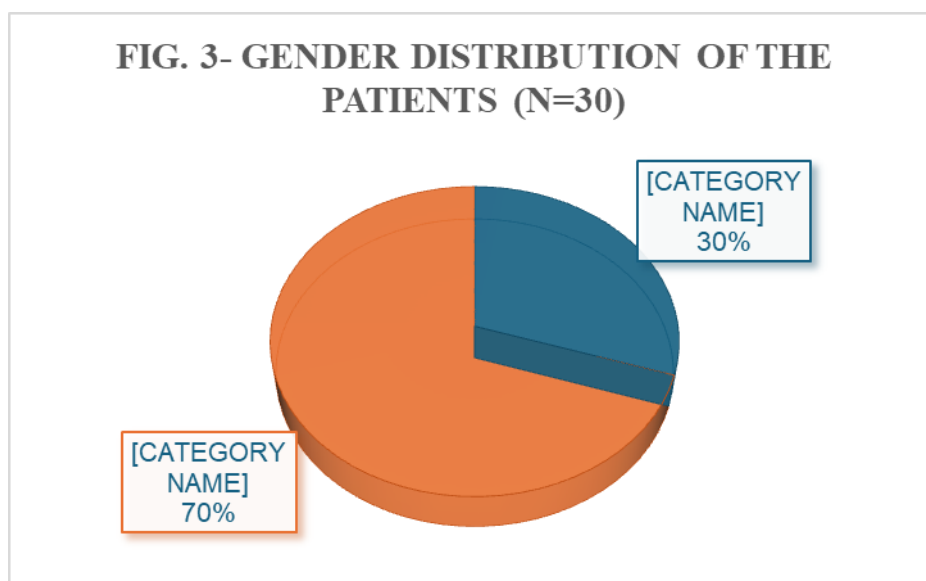
Figure 2: Study Flow Diagram.

Baseline Descriptive Statistics

A total of six variables were studied on the subjects – Sex, Age-group, Religion, Occupation, Types of Patient of recent injury, Baseline NRS Score and After NRS Score for the socio- demographic analysis of the Patient of recent injury.

Table 1: Gender distribution of the patients (n=30)

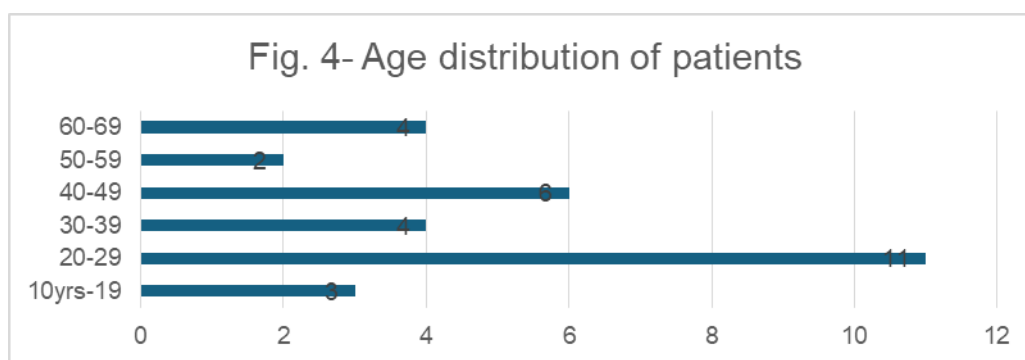
Sex	No. of Patient	Percentage
Female	9	30.0%
Male	21	70.0%



Out of 30 patients, 21 (70.0%) are males and 9 (30.0%) are females.

Table 2: Different age group (years) of Patients (n=30).

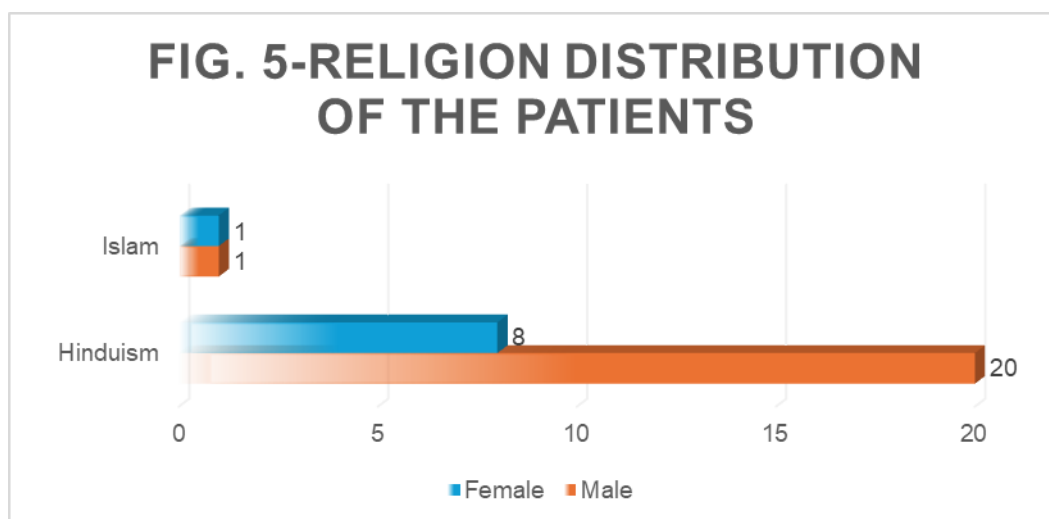
Age group (years)	No. of Patient	Percentage
10-19	3	10.00%
20-29	11	36.66%
30-39	4	13.33%
40-49	6	20.00%
50-59	2	06.66%
60-69	4	13.33%
Grand Total	30	100.00%



Out of 30 patients, 3 (10.00%) patient is in age group 10-19 years, 11 (36.66%) patients is in age group 20-29 years, 4 (13.33%) patients is in age group 30-39 years, 6 (20.00%) patients is in age group 40-49 years, 2 (06.66%) patients is in the age group 60-69 years.

Table 3: Distribution patients according to Religion.

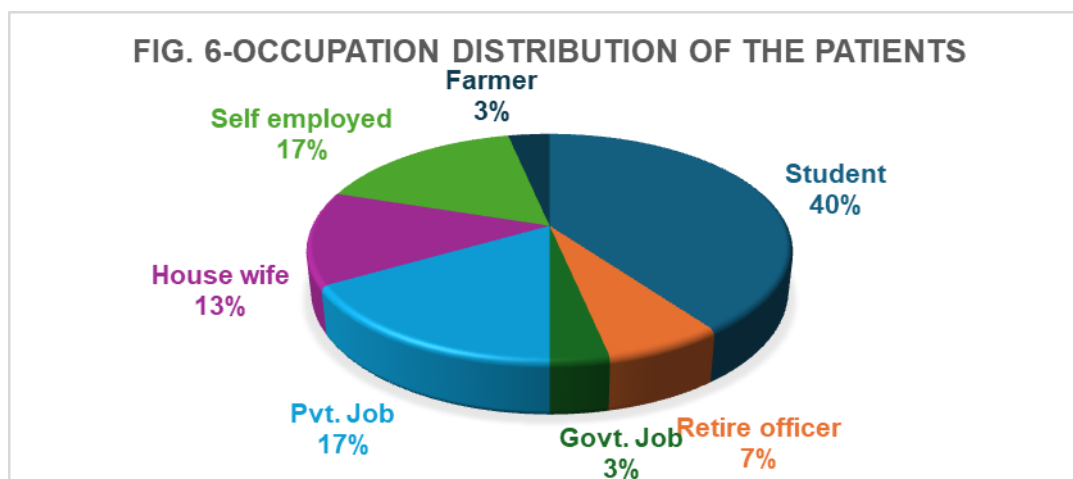
S.No.	Religion	Male	Female	Total	Percentage
1	Hinduism	20	8	28	93.33
2	Islam	1	1	2	06.66
3	Total	21	9	30	100



Out of 30 patients 28(93.33%) follow Hinduism, consisting of 20 males and 8 females, rest 2(10.0%) follow Islam consisting of 1 males and 1 female.

Table 4: Distribution patient according to their occupation.

S.No	Occupation	Number of Cases	Percentage
1	Student	12	40.00
2	Retire officer	2	06.66
3	Govt. Job	1	03.33
4	Pvt. Job	5	16.66
5	House wife	4	13.33
6	Self employed	5	16.66
7	Farmer	1	03.33
8	Total	30	100



Out of 30 patients , 12(40%) patients belongs to student group, 2(6.66%) patients are from Retire officers, 1(3.33%) patient from Govt. job, 5(16.66%) patients are from Pvt. Job, 4(13.33%) patients are House wife, 5(16.66%) patients belongs to Self-employed and 1(3.33%) patient was Farmer.

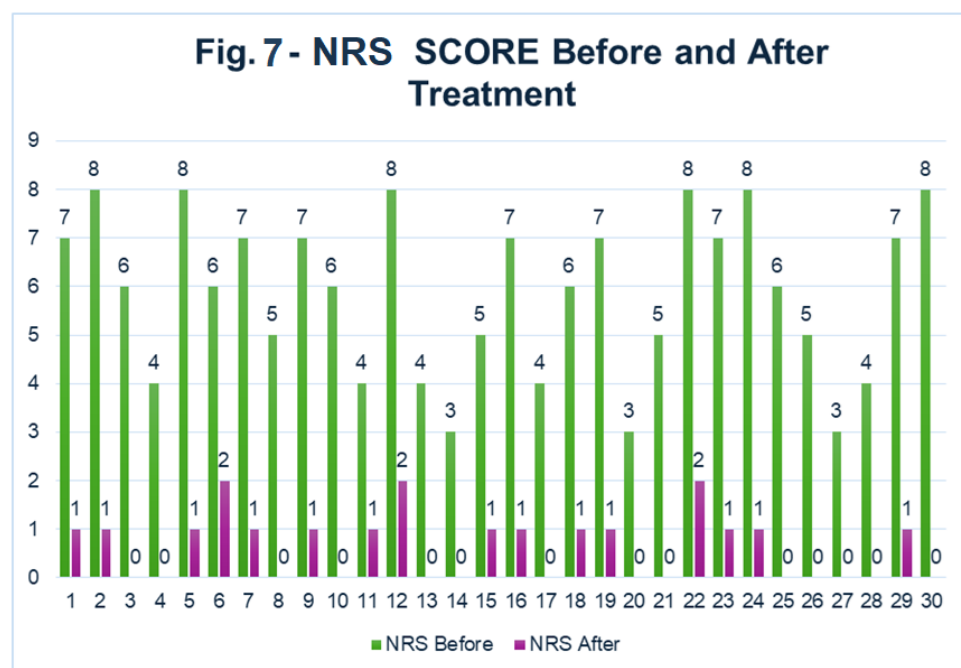
Table 5: Comparison of NRS score values before and after treatment.

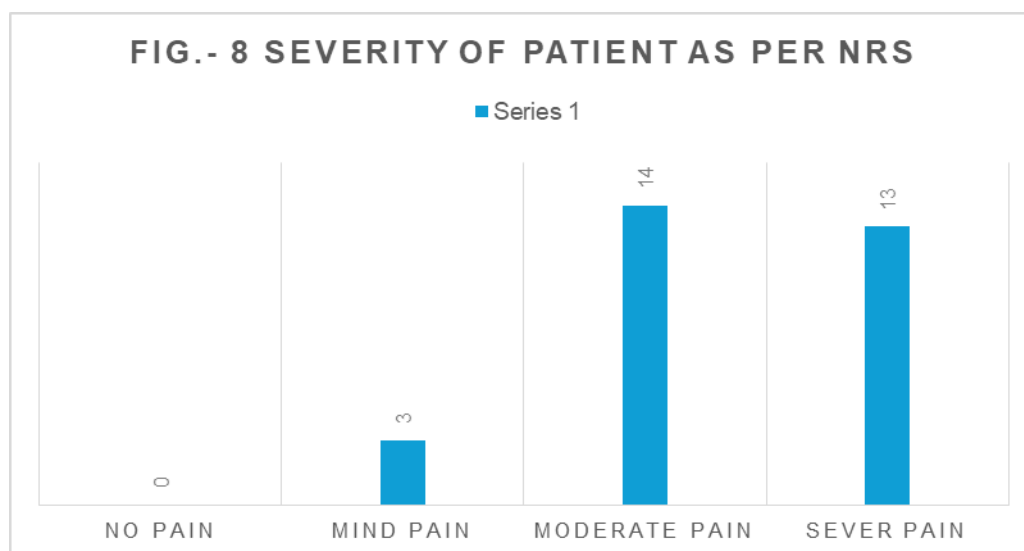
Case No.	NRS Before	NRS After
1	7	1
2	8	1
3	6	0

4	4	0
5	8	1
6	6	2
7	7	1
8	5	0
9	7	1
10	6	0
11	4	1
12	8	2
13	4	0
14	3	0
15	5	1
16	7	1
17	4	0
18	6	1
19	7	1
20	3	0
21	5	0
22	8	2
23	7	1
24	8	1
25	6	0
26	5	0
27	3	0
28	4	0
29	7	1
30	8	0

Table 6: Severity of Patients as per NRS scale.

S. No.	Severity	No. of patient
1	No pain	0
2	Mild pain	3
3	Moderate pain	14
4	Sever Pain	13
5	Total	30





Out of 30 patients, 13(43.33%) patients belongs to sever pain group, 14(46.66%) patients are from Moderate pain, 3(10.00%) patient from mild pain, and no patients with on pain group.

Statistical Analysis

The data of 30 subjects who had completed the study starting from the baseline to 6/7 days was subjected to inferential statistical analysis. The WURSS score of the same subjects were compared at the baseline and 6th/7th day respectively. Paired t-test was applied to determine the statistical significance of the change in WURSS scores of these patients.

Assessment of WURSS Scores at the baseline & 6th/7th day

Table 7: Comparison of mean values of NRS scores before and after treatment (n=30).

Mean NRS Score baseline	Mean NRS score after 6/7 days	Mean difference	Standard Deviation	Standard Error
5.86	0.63	5.23	1.356	0.247

DISCUSSION

Principal Findings

The **present study** focused on the qualitative phytochemical analysis of the *Arnica montana* Mother tincture procured from a GMP certified company. Various phytochemical tests using different reagents were performed on the sample to elicit the comparison of the active phytochemicals in the sample with that of the standard extract as mentioned in various studies.^[2,3,4,5] The results showed the presence of Terpenoids, flavonoids, Glycosides, phenolic compounds, fixed oils & fats, Phytosterols and flavonoids in *Arnica montana* Mother tincture. This **finding** is suggestive of the fact that same phytochemicals are present in the tested sample of *Arnica montana* Mother tincture with the alcohol strength of 59% v/v and other extracts of varying alcoholic strength. Hence, the tested sample met the qualitative phytochemical standards. These metabolites which are present in this formulation may also be retained in its ultra-high dilutions (successive potencies of *Arnica montana*) which might be responsible for its dynamic action.

A prospective, observational clinical study was carried out on 30 patients suffering from Acute upper respiratory tract infections to determine the effect of *Arnica montana* Mother Tincture (homoeopathic medicine) on Quality of Life.

Socio-demographic data were obtained for 30 patients after including them for the trial. Socio-demographic data discussed below:

Gender

Out of 30 patients, 21 (70.0%) are males and 9 (30.0%) are females.

Age

Out of 30 patients, 3 (10.00%) patient is in age group 10-19 years, 11 (36.66%) patients is in age group 20-29 years, 4 (13.33%) patients is in age group 30-39 years, 6 (20.00%) patients is in age group 40-49 years, 2 (06.66%) patients is in the age group 60-69 years.

Religion

Out of 30 patients 28(93.33%) follow Hinduism, consisting of 20 males and 8 females, rest 2(10.0%) follow Islam consisting of 1 males and 1 female.

Occupation

Out of 30 patients , 12(40%) patients belongs to student group, 2(6.66%) patients are from Retire officers, 1(3.33%) patient from Govt. job, 5(16.66%) patients are from Pvt. Job, 4(13.33%) patients are House wife, 5(16.66%) patients belongs to Self-employed and 1(3.33%) patient was Farmer.

Severity of disease

Out of 30 patients, 13(43.33%) patients belongs to sever pain group, 14(46.66%) patients are from Moderate pain, 3(10.00%) patient from mild pain, and no patients with on pain group.

NRS values at the baseline and after six/seven days of the intervention were also compared. Since, it was a single-arm study, NRS scoring was compared before and after treatment for these 30 patients. The NRS scores of patients (n=30) were analyzed before and after treatment. The mean NRS score at the baseline was 5.866 which reduced to 0.633 after treatment indicatingan improvement in the NRS of these patients from moderate to good. When these NRS scores were compared within the group before and after treatment by applying Student paired t test for comparison of the score at baseline and after receiving *A. montana* Q, the improvement was statistically significant [NRS: $t_{cal}=21.1$, $t_{crit}= 2.045$].

This indicates that *Arnica montana* Q played a significant role in improving the Quality of Life of the patients suffering recent injury.

Hence, **Null hypothesis** that “there is no significant effect of *Arnica montana* on Quality of Life of patients is rejected” and **Alternative hypothesis** that “*Arnica montana* Q has a significant effect on Quality of Life of patients is accepted”.

Comparison with other available studies

No clinical trials have been conducted with *A. montana* Q in recent injury where it has been used in the form of homoeopathic mother tincture.

Strengths of the study

- The results of the qualitative phytochemical analysis of the *A. montana* Mother tincture conformed with the other extracts prepared according to different pharmacopoeias.

- The clinical trial was an observational prospective one where the protocol was declared before the enrolment of the first patient. There is no evidence of a similar study conducted in homeopathy on the recent injury with Arnica as an intervention in the form of Mother tincture. The study included qualitative (NRS Score) aspects of recent injury.
- Before the enrolment, each patient was provided with informed consent detailing the study aims and objectives of the study with the complete right of the patient to withdraw from the study at any time.
- The scale used for the study (NRS) is the most common scale used for the assessment of the Quality of Life in patients with Pain.

Limitations of the study

- This was done keeping in mind the ethical considerations. Moreover, many patients with recent injury would not have opted for homeopathy when the standard treatment for the same is established.

Future prospects

A similar study can be conducted in the future with larger sample size. Moreover, a comparative study with the standard treatment can also be considered for more firm evidence. Phytochemical analysis of the different potencies of *Arnica montana* can be performed to compare the results with that of the Mother tincture. This might help in explaining the mechanism of action in ultra-high dilutions.

CONCLUSION

The results of the qualitative phytochemical analysis of the *Arnica montana* Mother tincture indicated the presence of secondary metabolites like Terpenoids, flavonoids, Glycosides, phenolic compounds, fixed oils & fats, Phytosterols and flavonoids which are responsible for its inflammatory action. Further research is necessary with the homeopathic dilutions of the plant to justify its mechanism of action. A single-arm, prospective observational study was conducted on patients with recent injury to determine the efficacy of *Arnica montana* Mother tincture on Quality of Life of these patients. NRS score differences at baseline and after treatment of 5th/7th day were statistically significant.

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