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EXTRACTION, PHYTOCHEMICAL ANALYSIS AND STUDY OF PHARMACOLOGICAL POTENTIAL OF *INULA RECEMOSA* EXTRACT

Ashutosh Dubey*, Jitendra Kumar Malik, Gyan Singh
P.K. University, Thanra, Shivpuri (M.P.)

***Correspondence Info:**
Ashutosh Dubey
P.K. University, Thanra, Shivpuri (M.P.)
Email:
ashutoshdubey1444@gmail.com

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ABSTRACT

The present study was undertaken to evaluate the extraction, phytochemical constituents, and pharmacological potential of the ethanolic extract of *Inula racemosa*. The plant extract was prepared using ethanol, yielding 10.52% w/w, indicating effective extraction of bioactive compounds. Preliminary phytochemical screening revealed the presence of important secondary metabolites such as flavonoids, phenols, glycosides, saponins, proteins, and carbohydrates, while alkaloids, tannins, diterpenes, and sterols were absent. Quantitative analysis showed appreciable amounts of total phenolic (0.652 mg/100 mg) and flavonoid content (0.741 mg/100 mg), suggesting significant antioxidant potential. The pharmacological evaluation demonstrated that the extract exhibited dose-dependent anti-inflammatory activity, though less potent than diclofenac sodium, with an IC_{50} value of 114.21 µg/ml. Antimicrobial studies showed moderate activity against *Streptococcus mutans* and *Escherichia coli*, with increasing zones of inhibition at higher concentrations. Furthermore, the extract displayed notable antidiabetic activity through α-amylase inhibition, with an IC_{50} value of 98.52 µg/ml compared to acarbose. The findings indicate that *Inula racemosa* possesses significant pharmacological properties, which may be attributed to its rich phytochemical composition. Although the activity is comparatively lower than standard drugs, the plant extract offers a promising natural alternative for therapeutic applications. Further studies are required to isolate active constituents and validate its efficacy through *in-vivo* models.

Keywords: *Inula racemosa*, Ethanolic extract, Phytochemical screening, Flavonoids, Phenolic compounds, Anti-inflammatory activity, Antimicrobial activity, Antidiabetic activity, IC_{50}, Medicinal plants.

INTRODUCTION

Medicinal plants have been widely used since ancient times as a primary source of therapeutic agents for the treatment and prevention of various diseases. In recent years, there has been a growing interest in herbal medicine due to its perceived safety, efficacy, and minimal side effects compared to synthetic drugs (Shakya, 2016).

A large proportion of the global population still depends on plant-based medicines for primary healthcare, emphasizing the importance of exploring phytochemical constituents and pharmacological potential of medicinal plants (Hemler and Hu, 2019).

Inula racemosa Hook. f., commonly known as Pushkarmool, is an important medicinal plant belonging to the family Asteraceae. It is primarily distributed in the temperate and sub-

alpine regions of the Western Himalayas, including areas of Kashmir, Himachal Pradesh, and Uttarakhand. Traditionally, the plant has been widely used in Ayurveda, Unani, and Tibetan systems of medicine for the treatment of respiratory disorders such as asthma, bronchitis, and cough, as well as cardiovascular diseases, skin disorders, and digestive ailments (Jaiswal *et al.,* 2022).

The pharmacological importance of *Inula racemosa* is mainly attributed to its rich phytochemical composition. The plant contains a variety of bioactive constituents such as sesquiterpene lactones (including alantolactone and isoalantolactone), flavonoids, terpenoids, and phenolic compounds. These compounds are responsible for its diverse biological activities, including anti-inflammatory, antioxidant, antimicrobial, cardioprotective, hepatoprotective, and anti-allergic effects. The presence of such phytoconstituents makes the plant a promising candidate for the development of novel therapeutic agents (Jaiswal *et al.,* 2022).

Phytochemical analysis plays a crucial role in identifying the active constituents present in plant extracts and correlating them with their pharmacological activities. Various extraction methods such as Soxhlet extraction, maceration, and solvent extraction are commonly employed to isolate bioactive compounds from plant materials. These extracts are then subjected to qualitative and quantitative phytochemical screening to determine the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and other secondary metabolites (Altemimi *et al.,* 2017).

The pharmacological evaluation of *Inula racemosa* extracts has revealed a wide range of biological activities. Studies have demonstrated its effectiveness in reducing inflammation, protecting against oxidative stress, and exhibiting antimicrobial and cytotoxic properties. Its cardioprotective and anti-asthmatic effects have been well documented in traditional and modern research, highlighting its therapeutic significance (Patel *et al.,* 2021).

Despite its extensive traditional use and pharmacological potential, *Inula racemosa* is considered a critically endangered species due to overexploitation and habitat loss. There is a need for systematic scientific investigation to validate its medicinal properties and promote its conservation and sustainable utilization

Hence, the present study is focused on the extraction, phytochemical analysis, and evaluation of the pharmacological potential of *Inula racemosa* extract. This research aims to provide scientific evidence supporting its traditional uses and to explore its potential as a source of novel therapeutic agents.

MATERIALS AND METHODS

Materials

The materials used in the study included gallic acid and quercetin as standard compounds for the estimation of total phenolic and flavonoid content, respectively. Ethanol and methanol were used as solvents for extraction and preparation of standard and sample solutions. Folin–ciocalteu reagent and sodium carbonate were used for phenolic content determination, while aluminum chloride ($AlCl_3$) was used for flavonoid estimation. Distilled water was used for dilution purposes, and all reagents employed were of analytical grade.

Methods

Extraction by ultrasonic-assisted extraction process

50 gm of dried powdered roots of *Inula racemosa* has been extracted with ethanol solvent using ultrasonic-assisted extraction process for 24 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007).

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Estimation of total phenol content

The total phenolic content of the extract was determined using the modified Folin–Ciocalteu method as described by Mishra et al. (2017). In this method, 10 mg of gallic acid was dissolved in 10 ml of methanol to prepare the standard stock solution, from which various concentrations ranging from 10–50 µg/ml were prepared. For sample preparation, 10 mg of the dried extract was dissolved in 10 ml of methanol and filtered to obtain a clear solution, and 2 ml of this extract (1 mg/ml) was used for analysis.

The procedure involved mixing 2 ml of the extract or standard solution with 1 ml of Folin–Ciocalteu reagent (previously diluted with distilled water in a ratio of 1:10 v/v) and 1 ml of sodium carbonate solution (7.5 g/L). The reaction mixture was vortexed for about 15 seconds and then allowed to stand for 10 minutes for the development of a blue-colored complex. The absorbance of the resulting solution was measured at 765 nm using a UV-visible spectrophotometer. The total phenolic content was then calculated using the calibration curve of gallic acid and expressed as mg of gallic acid equivalents per 100 mg of extract.

Estimation of total flavonoids content

The total flavonoid content of the extract was determined using the aluminum chloride colorimetric method as described by Mishra *et al.*, (2017). In this method, 10 mg of quercetin was dissolved in 10 ml of methanol to prepare the standard stock solution, and further dilutions ranging from 5–25 µg/ml were prepared. For sample preparation, 10 mg of the dried extract was dissolved in 10 ml of methanol and filtered to obtain a clear solution, and 3 ml of this extract (1 mg/ml) was used for analysis.

The procedure involved adding 1 ml of 2% aluminum chloride ($AlCl_3$) solution to 3 ml of the extract or each standard solution. The

reaction mixture was allowed to stand for 15 minutes at room temperature for the formation of a yellow-colored complex. The absorbance was then measured at 420 nm using a UV-visible spectrophotometer. The total flavonoid content was calculated using the calibration curve of quercetin and expressed as mg of quercetin equivalents per 100 mg of extract.

In vitro* anti-inflammatory activity of ethanolic extract of *Inula racemosa

Protein denaturation assay was done according to the method described by Gambhire *et al.* (2009), with some modifications as described in Gunathilake *et al.,* (2018). Diclofenac sodium, a powerful non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisted of 0.2 mL of 1% bovine albumin, 4.8 mL of phosphate buffered saline (PBS, pH 6.4), and 0.2 mL of extract (10-50 µg/mL), and the mixture was mixed, and was incubated in a water bath (37◦C) for 15 min, and then the reaction mixture was heated at 70°C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer. Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition of denaturation} = (A1 - A2/A1) \times 100$$

Where A1 = absorption of the control sample, and A2 = absorption of the test sample

In vitro* antimicrobial activity of ethanolic extract of *Inula racemosa

The well diffusion method was used to determine the antimicrobial activity of the ethanolic extract prepared from of *Inula racemosa* using standard procedure (Bauer *et al.,* 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37ºC for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

In vitro* anti-diabetic activity of ethanolic extract of *Inula racemosa

Inhibition of alpha amylase enzyme

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100-500µg/ml were prepared in methanol. 10 mg of phytosome was extracted with 10 ml methanol. 500 µl of this solution was used for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (25-150µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube (Kidane *et al.,* 2018). The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

The present study focused on the extraction, phytochemical evaluation, and pharmacological potential of the ethanolic extract of *Inula racemosa*. The percentage yield of the ethanolic extract was found to be 10.52% w/w, indicating efficient extraction of bioactive constituents using ethanol as a solvent. Ethanol is known to extract a wide range of polar and moderately non-polar compounds, which justifies the appreciable yield obtained.

Preliminary phytochemical screening revealed the presence of important secondary metabolites such as glycosides, flavonoids, saponins, phenols, proteins, and carbohydrates, while alkaloids, diterpenes, tannins, and sterols were absent. The presence of flavonoids and phenolic compounds is particularly significant, as these compounds are well known for their antioxidant, anti-inflammatory, and antimicrobial activities. The absence of certain constituents may be attributed to the solvent specificity or geographical variation of the plant.

The quantitative estimation showed that the total phenolic content (0.652 mg/100 mg) and total flavonoid content (0.741 mg/100 mg) were present in considerable amounts. These phytoconstituents play a important role in scavenging free radicals and contribute to the therapeutic potential of the plant extract.

The anti-inflammatory activity, assessed by percentage inhibition, demonstrated that the ethanolic extract exhibited dose-dependent inhibition, although slightly lower compared to the standard drug diclofenac sodium. The IC_{50} value of the extract (114.21 µg/ml) was higher than that of diclofenac (63.93 µg/ml), indicating moderate anti-inflammatory activity. This activity may be attributed to the presence of flavonoids and phenolic compounds, which are known to inhibit inflammatory mediators.

The antimicrobial activity results showed that the ethanolic extract exhibited significant inhibitory effects against *Streptococcus mutans* and *Escherichia coli*. However, the zone of inhibition was lower compared to standard antibiotics such as ofloxacin and ciprofloxacin. The activity increased with concentration, confirming a dose-dependent antimicrobial effect**.** This antimicrobial potential may be due to the presence of bioactive compounds such as flavonoids, saponins, and phenolics.

In the antidiabetic study, the extract showed moderate α-amylase inhibitory activity compared to the standard drug acarbose. The IC_{50} value of the extract (98.52 µg/ml) was higher than acarbose (42.20 µg/ml), indicating comparatively lower potency. The extract still demonstrated significant inhibition, suggesting its potential role in managing postprandial hyperglycemia.

The study confirms that *Inula racemosa* possesses notable pharmacological activities, including anti-inflammatory, antimicrobial, and antidiabetic effects. These activities are largely attributed to the presence of phenolic and flavonoid compounds. Although the extract is less potent than standard drugs, it offers a natural and safer alternative with fewer side effects**,** supporting its traditional medicinal use.

Table 1: % Yield of *Inula racemosa*

S. No.	Extract	Weight of extract	% Yield (w/w)
1.	Ethanolic	5.26	10.52

Table 2: Result of phytochemical screening of ethanolic extract of *Inula racemosa*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Wagner's test:	-Ve
	Hager's test:	-Ve
2.	**Glycosides**	
	Conc. H_2SO_4 test	+Ve
3.	**Flavonoids**	
	Alkaline reagent test:	+Ve
	Lead acetate test:	+Ve
4.	**Saponins**	
	Froth test:	+Ve
5.	**Phenol**	
	Ferric chloride test:	+Ve
	Folin ciocalteu test	+Ve
6.	**Proteins**	
	Xanthoproteic test:	+Ve
7.	**Carbohydrate**	
	Fehling's test:	+Ve
	Benedict's test:	-Ve
8.	**Diterpenes**	
	Copper acetate test:	-Ve
9.	**Tanins**	
	Gelatin test	-Ve
10.	**Sterols**	
	Salkowski test	-Ve

[+Ve= Present; -Ve= Absent]

Table 3: Total phenol and flavonoid content of *Inula racemosa*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Ethanolic extract	0.652	0.741

Table 4: % Inhibition of Diclofenac sodium and ethanolic extract of *Inula racemosa*

Concentration (µg/ml)	% Inhibition	
	Diclofenac sodium	***Inula racemosa* extract**
100	51.67	45.74
200	64.32	59.13
300	73.79	67.98
400	81.64	70.52
500	90.25	78.45
IC 50 value	**63.93**	**114.21**

Table 5: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	**20 µg/ml**	**30 µg/ml**
1	Ofloxacin	*Streptococcus mutans*	12±0.15	15±0.13	17±0.19
2.	Ciprofloxacin	*Escherichia coli*	22±0.47	26±0.47	30±0.47

***Average of three determination, Mean ± SD**

Table 6: Antimicrobial activity of ethanolic extract of *Inula racemosa* against selected microbes

S. No.	Microbes	Zone of Inhibition (nm)		
		25mg/ml	**50 mg/ml**	**100 mg/ml**
1	*Streptococcus mutans*	9±0.47	12±0.94	15±0.86
2	*Escherichia coli*	8±0.57	10±0.74	14±0.5

***Average of three determination, Mean ± SD**

Table 7: % Inhibition of acarbose and ethanolic extract of *Inula racemosa*

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	***Inula racemosa* extract**
1	25	40.78	18.96
2	50	55.39	41.68
3	75	64.12	46.71
4	100	72.98	50.98
5	125	80.74	54.19
6	150	89.25	60.74
IC_{50} value (µg/ml)		**42.20**	**98.52**

CONCLUSION

The study confirms that *Inula racemosa* extract possesses significant phytochemical constituents, particularly phenolics and flavonoids, which contribute to its pharmacological activities. The extract exhibited moderate anti-inflammatory, antimicrobial, and antidiabetic effects in a dose-dependent manner. The findings support its potential as a natural therapeutic agent, though further studies are required for detailed evaluation and application.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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